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ARTIFICIAL CARIES PRODUCED BY STREPTOCOCCUS VIRIDANS[1]

CAROLYN HAMMOND, M.A., AND RUTH TUNNICLIFF, M.D.

The Foundation for Dental Research of the Chicago College of Dental Surgery and the John McCormick Institute for Infectious Diseases, Chicago

Tunnicliff and Hammond (1, 2) have described the isolation of a greening streptococcus from dental caries which in its smooth phase (*fig. 1*) grew as a diplococcus or in short chains (*fig. 2*) and produced acid (pH 4.4–4.8) in 1 per cent dextrose broth, pH 7.0. In its rough phase (*fig. 3*) this streptococcus showed bacillary and coiled forms, crescents, and straight and undulating filaments (*figs. 4–7*) and gave a pH value of 5.2–6.0 in 1 per cent dextrose broth, pH 7.0. These forms appeared to correspond to the cocci, rods, and "tortuous threads" described by Miller (3) in dentinal tubules of carious teeth and considered by him to be due to a mixed infection. He observed that cocci always predominated and might be the only organism present in the tubules. Cocci mixed with rods or filaments were observed in separate tubules and might also be mixed in the same tubule. We (1, 2) examined sections from 7 carious teeth and found cocci alone in those with slight caries, whereas cocci associated with bacillary and undulating filamentous forms were observed where there was more extensive destruction of dentin. Cocci always predominated and were seen deeper in the tubules than bacilli and filaments. Crescents were occasionally associated with the bacilli.

Artificial caries has been produced with both streptococci and lactobacilli by numerous investigators (3, 4, 5, 6, 7, 8). However, none of the descriptions and illustrations of artificial caries showing organisms in dentinal tubules mention, or show, tubules containing filaments or crescent forms. The illustrations show either cocci or bacilli or both. Since we had observed crescents and filaments in our rough

[1] Presented at the 17th General Meeting of the International Association for Dental Research, Cleveland, Ohio, March 18–19, 1939. (*J. D. Res.* **18:** 281, 1939.)

cultures, we decided to attempt to produce caries artificially in order to see if we could find these forms in the tubules.

It was difficult to decide on a method of sterilizing the extracted teeth. Various procedures were tried at the time when we were attempting to sterilize only the outside of the tooth in order to leave viable any organisms in the pulp (9). The method found satisfactory then was not applicable in this case as it was essential that all organisms inside as well as outside the tooth be killed. We decided on autoclaving as the surest method. We are aware that this method may produce a change in the proteins of the tooth, but any effective method of sterilization will produce such a change. The ultraviolet radiation recommended by Pincus (10) was not thought feasible since the rays penetrate to only 1 mm. in protein and hence would hardly penetrate enamel sufficiently to destroy any organisms drawn into the pulp during extraction (9, 11). Furthermore, since the extracted teeth remained 3 to 4 months in a 1 per cent dextrose broth of an average pH of 4.4, protein changes would probably take place very soon after immersion, regardless of the method of sterilization.

The percentage of sugar in the medium apparently has no effect on the amount of acid produced by the organisms. We inoculated with the streptococcus a series of tubes of plain broth and broth containing 1, 2, 5, 10, 15, 20, 25 and 30 per cent dextrose. The pH was tested by means of the Coleman glass electrode at 1 and 2 week intervals. The plain broth showed growth but the pH remained neutral. The pH of all the others varied by only 0.3. It ranged from 4.0–4.3.

Ten freshly extracted, apparently intact incisors, canines, and premolars were used in the experiment. The teeth were examined thoroughly with an explorer and no caries was found. They were scrubbed with soap and water, dried, placed individually in test tubes, and autoclaved at 15 lbs. pressure for 25 minutes (*figs. 8, 9*). They were then dipped in sterile pink dental wax in such a way that the entire root and $\frac{2}{3}$ of the crown were covered with a fairly thick coating of wax. This left $\frac{1}{3}$ of the crown exposed to the action of the acid produced by the bacteria. The wax-covered tooth was next placed in a tube containing 10 cc. of 1 per cent dextrose broth, pH 7.0, and was incubated 24 hours to insure sterility. Then it was inoculated with organisms picked from a smooth colony of *S. viridans* (*fig. 1*) isolated from carious dentin. A smear made from such a colony revealed only cocci in pairs or an occasional short chain (*fig. 2*). At first fresh broth was put on the teeth every other day. However, since the pH rarely went lower than 4.8–5.0, the broth was allowed to remain in the tubes a week. The pH ranged

from 4.2–4.6 under this weekly routine. At each change of broth blood agar plates were streaked to determine possible contamination or dissociation. Smears were made to observe any morphologic changes. The pH was obtained colorimetrically using methyl red and LaMotte yellow as indicators. The samples were checked with Coleman's glass electrode several times during the course of the experiment. Throughout the experiment all cultures remained free from any contamination.

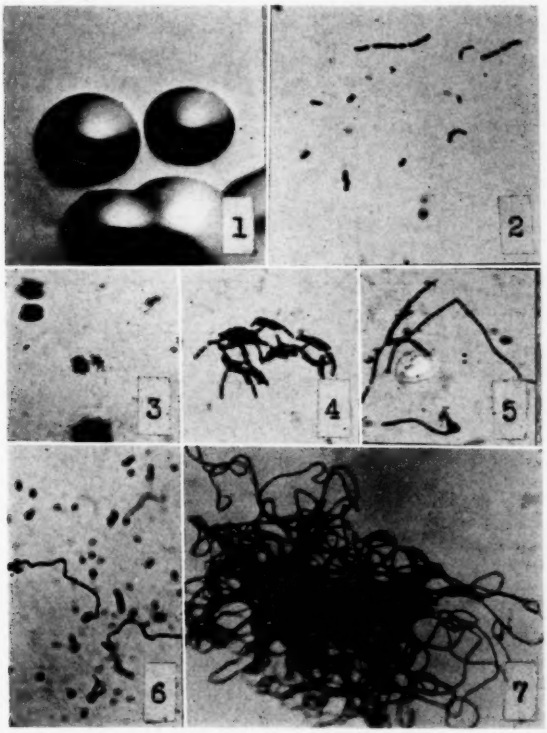
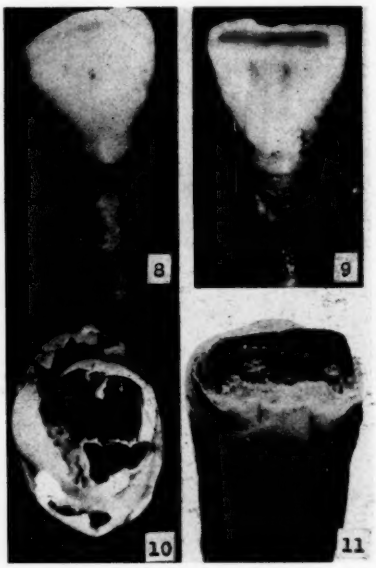


FIG. 1. Smooth colony of *S. viridans* isolated from carious dentin. ×30.
FIG. 2. Diplococci in pairs and chains from smooth colony of *S. viridans*. ×1,200.
FIG. 3. Rough colony of *S. viridans* isolated from carious dentin. ×30.
FIGS. 4–7. Pleomorphic forms of streptococci found in rough colonies. ×1,200.

Teeth were removed and sectioned at the following intervals: 14 days, 23 days, 31 days, 3 months, 4 months, and 6 months. After removal from the broth the teeth were washed in water and the wax was removed. They were then placed in 10 per cent formalin for 24 hours, decalcified in 5 per cent nitric acid, embedded in celloidin, sectioned, and stained with hematoxylin-eosin and Gram-Weigert stains.

Etching of the enamel was grossly visible in some teeth as early as 14 days but on sectioning no organisms were found either in dentin or pulp. At the end of 3 months all teeth showed chalky spots on the enamel. Three teeth were removed at this interval and sectioned. Only one showed organisms, cocci, in the dentinal tubules. However, at the end of 4 months all teeth showed extensive decalcification of the enamel (*figs. 10, 11*) and cocci, bacilli, crescents and filaments



FIGS. 8–9. Intact teeth after autoclaving.

FIGS. 10–11. Same teeth 6 months later showing wax covering, enamel destruction and exposed dentin.

were found in the dentinal tubules (*figs. 13–17*). In one instance a "liquefaction focus" (*fig. 17*) was observed, consisting of circumscribed distention of a tubule containing only cocci, and destruction of the surrounding dentin.

The predominance of bacilli and filaments was a little confusing since the broth cultures, when streaked on blood agar, yielded no definitely rough colonies. Possibly anaerobic conditions in the tubules were favorable to dissociation, since aerobic cultures show-

ing diplococcus forms only, when sealed in a flattened capillary pipet, grew out into filaments. At the end of the second month all cultures showed morphologically some rough features. Rods and occasional filaments were observed. However, on blood agar only smooth colonies were formed.

A second series of 10 teeth was then treated by the same technic as the first group. After being placed in broth they were inoculated

FIG. 12. Section of tooth showing artificially produced caries with tubules invaded by bacteria and "liquefaction foci." Gram-Weigert stain. ×58.

with a well stabilized smooth strain of *S. viridans* which showed only diplococci and had been isolated from carious dentin. The broth on these teeth was changed biweekly as it was thought that possibly the 7 day period of growth was an aid to dissociation in the first series. By increasing the wax-covered surface the amount of enamel exposed to the action of the acid was cut down to a very small area on the cusp.

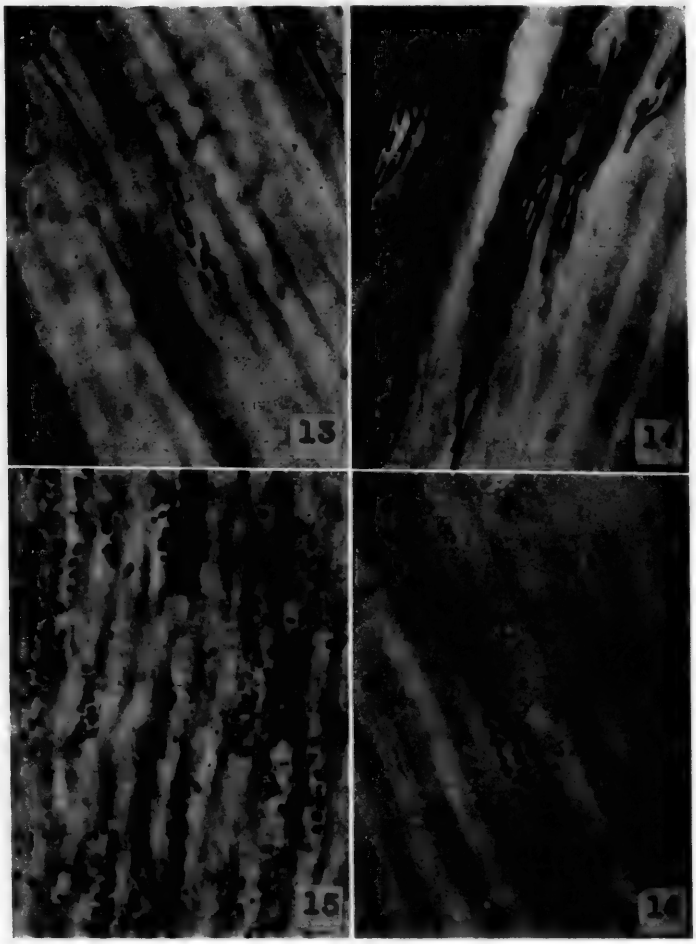


FIG. 13. Artificial caries. Bacilli in tubules. Gram-Weigert stain. ×1,600.
FIG. 14. Artificial caries. Filaments in tubules. Gram-Weigert stain. ×1,600.
FIG. 15. Artificial caries. Tubules contain only cocci. Gram-Weigert stain. ×1,600.
FIG. 16. Artificial caries. Crescents in tubules. Gram-Weigert stain. ×1,600.

At the end of 3 months 2 teeth in streptococcus cultures were removed for sectioning. Although the tubules were not extensively invaded at this time, some contained bacteria. Coccus forms were

the only organisms observed. At the end of 4 months 4 teeth showed cocci alone invading both tubules and pulps (*fig. 15*). No other forms were found probably due to the better stabilization of the organism. On 2 of these teeth plaques (*fig. 18*) were demonstrated which had formed on the outside of the dentin apparently by matted cocci. No bacteria were seen invading the tubules under the plaques.

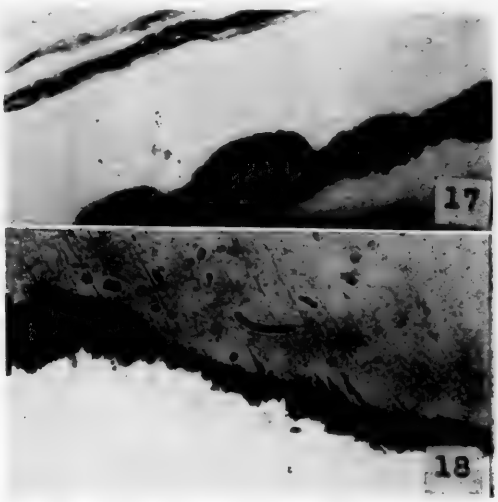


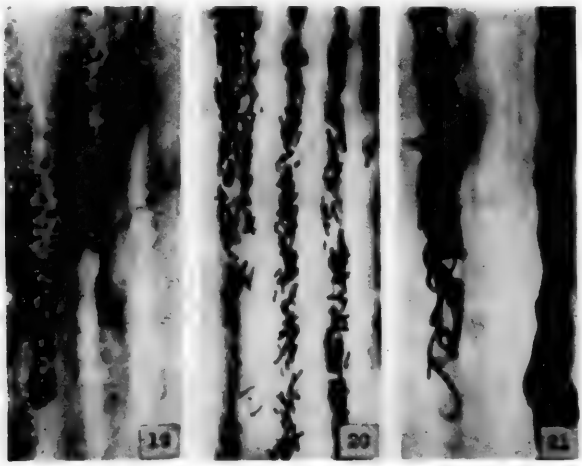
FIG. 17. Artificial caries. "Liquefaction focus" containing only cocci. Gram-Weigert stain. ×1,200.

FIG. 18. Artificially produced plaque made up of cocci on dentin. Gram-Weigert stain. ×265.

As controls 2 teeth were placed in uninoculated 1 per cent dextrose broth the pH of which had been artificially lowered to 4.0 with lactic acid. At the end of 3 and 4 months the teeth in lactic acid broth showed considerable enamel destruction but no organisms were seen in the tubules.

Two wax-covered teeth were placed in 1 per cent dextrose broth and inoculated with a freshly isolated strain of *B. coli*. This organism did not produce as much acid as the streptococcus, the average pH being 4.8. A slightly longer time was needed to produce the carious lesion, 4½ months being the shortest period of time before decalcifica-

tion of the enamel and invasion of the dentinal tubules by the organism occurred. As far as we were able to determine, the lesion differed in no respect from that produced by the streptococcus in its external appearance. The tubules were found full of rod-shaped organisms. No cocci, filaments or crescents were observed.



FIGS. 19, 20, 21. Cocci, bacilli and filaments in dentinal tubules in natural caries. ×1,600. [From Kronfeld, R., *Histopathology of the Teeth*, 2nd Ed. (Philadelphia, Lea and Febiger: 1939) p. 130.]

SUMMARY

Twenty freshly extracted, apparently intact incisors, canines, and premolars were autoclaved, dipped in melted, sterile dental wax in order to cover the entire root and part of the crown, and placed in 1 per cent dextrose broth, pH 7.0. 16 teeth were incubated in broth inoculated with a smooth colony of *S. viridans* isolated from carious dentin containing only coccus forms. By placing fresh broth on the teeth weekly or biweekly an average pH of 4.2 was maintained. Etching of the enamel was observed as early as 14 days after the beginning of the experiment. Teeth were removed, decalcified, sectioned and stained with hematoxylin-eosin and Gram-Weigert stains at 14, 23, 31 days and 3, 4 and 6 months. Three months was the shortest time before organisms were seen in the dentinal tubules,

when cocci alone were found. This is similar to the findings in early natural caries. After 4 months' incubation the tubules contained cocci, bacilli, crescents and filaments similar to those seen in more advanced natural caries. As controls 2 teeth were placed in 1 per cent dextrose broth the pH of which had been artificially lowered to 4.0 with lactic acid and 2 teeth were placed in 1 per cent dextrose broth inoculated with *B. coli*. After 3 months the lactic acid controls showed enamel destruction but no invasion of the tubules by bacteria was observed. *B. coli* producing a pH of 4.8 decalcified the enamel and invaded the tubules in which only bacilli were found.

ADDENDUM

Since this paper was originally written, an article by M. L. Snyder has been published (*J. D. Res.* **18:** 497, 1939) in which he asserts that we were studying impure cultures in our report on the rough and smooth phases of greening streptococci (J. A. D. A., **25:** 1046, 1938) because we isolated them from glucose brain broth in which "streptococci always present will grow readily but will shortly decrease in numbers under the acid produced and will be overgrown by the lactobacilli if present". Since the R and S phases of the streptococci, as stated by us, grow in 1 per cent dextrose, pH 4.4–5.0, and live 1 week or more in this acid medium, it is not strange that they were isolated from dextrose brain broth medium on blood agar. Both phases were repeatedly replated over a period of 3 months without showing any spontaneous change in morphology or colony formation indicating mixed cultures.

Streptococci were not "isolated from bacilli" as stated by Snyder, but bacillary forms were changed to streptococci over a period of weeks, a common observation in the dissociation of streptococci. The reason streptococci did not appear in the cultures of lactobacilli is probably that, so far, streptococci, stabilized as such, have not been dissociated from lactobacilli. Snyder omitted that streptococci in smooth colonies were also changed to bacilli in rough colonies. These changes from streptococci to bacilli and bacilli to streptococci have also been observed on the warm stage (*J. D. Res.* **18:** 177, 1939).

Snyder then takes part of a statement out of the context and critizes it as our conclusion after the study of only 14 streptococcus cultures. The whole statement, "However, the fact that, in natural caries, cocci are the predominating and often the only organism present in tubules indicates that the invading organism in dental caries is a coccus, whatever the predisposing factors", was not based on our streptococcus study only, but on a summary of the observations of Miller, Hartzell and Henrici, Clarke, Maclean and Anderson and Rettger.

REFERENCES TO LITERATURE

(1) Tunnicliff, R. and Hammond, C., *J. A. D. A.* **25:** 1046, 1938.
(2) Tunnicliff, R. and Hammond, C., *Arch. Path.* **26:** 61, 1938.
(3) Miller, W. D., *Die Microorganismen der Mundhöhle* (Leipsig, G. Thieme: 1889).

(4) McIntosh, J., James, W. W. and Lazarus-Barlow, P., *Brit. Dent. J.* **42:** 728, 1922.
(5) Rodriguez, F. E., *Military D. J.* **5:** 199, 1922.
(6) Clarke, J. K., *Brit. J. Exper. Path.* **5:** 141, 1924.
(7) Bunting, R. W. and Palmerlee, F., *J. A. D. A.* **12:** 381, 1925.
(8) Maclean, I. H., *Brit. D. J.* **48:** 579, 1927.
(9) Tunnicliff, R. and Hammond, C., *J. A. D. A.* **24:** 1663, 1937.
(10) Pincus, P., *Brit. D. J.* **66:** 350, 1939.
(11) Fish, E. W. and MacLean, I., *Brit. D. J.* **61:** 336, 1936.

THE IMPORTANCE OF A DETAILED CLINICAL EXAMINATION OF CARIOUS LESIONS

REIDAR F. SOGNNAES[1]

Forsyth Dental Infirmary for Children, Boston, Mass.

For clinical study and treatment of disease it is essential to establish unfailing diagnostic methods and universally accepted definitions of symptoms. As far as dental caries is concerned, the text-books do not associate this disease with any particular diagnostic difficulties. However, Gythfeldt (1) has shown that an examination of the caries frequency in a group of children gave a surprisingly great discrepancy in the findings, when carried out by several examiners, using the same diagnostic procedure. This indicates that certain manifestations of caries are liable to be overlooked, possibly due to a varying conception of early symptoms of the disease.

In addition to this source of error it is unfortunate that information regarding the caries frequency in different countries, communities, and smaller groups of individuals is not based on standardized diagnostic methods. Although the statistical evaluation of caries-records has been greatly improved by the modified caries-index worked out by Bodecker (2, 3), an accurate comparison of the results is not possible unless the examinations *per se* are carried out by comparable diagnostic methods. Thus the questions arise, firstly, to what extent do the clinical findings vary when different diagnostic procedures are employed and, secondly, what type of lesions are most liable to be overlooked.

PROCEDURE

In order to obtain some information regarding these questions a study was made of 32 infirmary out-patients. The average age was 9 years, the age range 4–13. Thus the deciduous as well as the permanent dentitions were represented in the group studied.

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[1] This manuscript was completed during the author's tenure as a Carnegie Fellow in Dentistry at the School of Medicine and Dentistry, The University of Rochester, Rochester, N. Y.

The dental examination was carried out in 4 progressive steps by the following diagnostic aids:

Procedure A: Mirror and explorer.
Procedure B: Aided by drying of the teeth.
Procedure C: Aided by cleaning and drying.
Procedure D: Aided finally by radiographic diagnosis.

Approximately 1½ hours were devoted to the examination of each child.

TABLE I

Carious teeth as found by the first and the complete examination

NUMBER OF CHILDREN	NUMBER OF TEETH EXAMINED			PROCEDURE	NUMBER OF CARIOUS TEETH					
					Deciduous teeth		Permanent teeth		All teeth	
	Deciduous	Permanent	In all		Number carious	Per child	Number carious	Per child	Number carious	Per child
32	308	449	757	Mirror & explorer	119 (39%)	3.7	166 (37%)	5.2	285 (38%)	8.9
				All proc. combined	164 (53%)	5.1	246 (55%)	7.7	410 (54%)	12.8

TABLE II

Carious surfaces as found by the four steps of the examination

PROCEDURE	NUMBER OF TOOTH-SURFACES					
	Deciduous teeth		Permanent teeth		All teeth	
	Surfaces examined	Carious surfaces	Surfaces examined	Carious surfaces	Surfaces examined	Carious surfaces
		no. (per cent)		*no. (per cent)*		*no. (per cent)*
A	1401	320(22.8)	2006	331(16.5)	3407	651(19.1)
A, B	1401	335(23.9)	2006	438(21.7)	3407	773(22.7)
A, B, C	1401	349(24.9)	2006	488(24.2)	3407	837(24.6)
A, B, C, D	1401	364(26.0)	2006	515(25.6)	3407	879(25.8)

RESULTS

The first examination with mirror and explorer alone (procedure A) left a considerable percentage of the actual carious teeth to be disclosed by the additional procedures (B, C, D), as is evident from Table I. More detailed information is given in Table II. A gradual increase is observed in the number of carious tooth surfaces that were recorded by the successive steps of examination.

Considering the total number of affected surfaces as 100 per cent,

the relative importance of each step of the examination is indicated in Table III. It will be seen that nearly $\frac{1}{4}$ of the total number of carious surfaces would have escaped notice if the additional procedures B, C, and D had not been included. The drying and cleaning of the teeth (procedures B and C) proved to be of special importance in the detection of a great many lesions in the permanent dentition. The reason for this was brought out when a study was next made of the

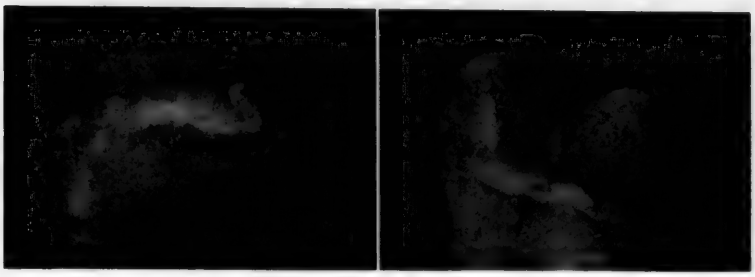

FIG. 1. "Pre-caries" (initial smooth-surface lesions). First permanent molar. Left: mesial surface, Right: lingual surface.

TABLE III

The relative importance of the different diagnostic procedures

PROCEDURE	DECIDUOUS TEETH		PERMANENT TEETH		ALL TEETH	
	Carious surfaces	Percent. of all car. s.	Carious surfaces	Percent. of all car. s.	Carious surfaces	Percent. of all car. s.
A	320	88.0	331	64.3	651	74.2
A, B	335(+15)	92.1(+4.1)	438(+107)	85.1(+20.8)	773(+122)	88.0(13.8)
A, B, C	349(+14)	95.1(+3.8)	488(+50)	94.8(+9.7)	837(+64)	95.3(+7.3)
A, B, C, D	364(+15)	100. (+4.1)	515(+27)	100. (+5.2)	879(+42)	100. (+4.7)

type of lesions disclosed by the different diagnostic procedures. Carious cavities and pit and fissure lesions were largely detected by procedure A according to the traditional "catch of the explorer". Cavities located in the approximal surfaces were readily observed in the radiograms (procedure D), when not revealed by the explorer. However, the remaining lesions, 20 per cent of all recorded, were disclosed only as a consequence of a detailed study of the cleaned and dried teeth. Those were enamel lesions located on the smooth surfaces of the teeth. They may be divided into 2 types according to the ad-

vancement of the enamel disintegration. In one, where the decalcification had advanced far enough to cause a slight roughness in the surface, the diagnosis could be made by drying of the enamel, followed by testing of the surface with a probe.[2] The other type of lesion could not be detected by the explorer as no roughness was noticed on the surface. However, by careful naked-eye examination of the cleaned and dried teeth (procedure C) these lesions manifested themselves as slight changes in the translucency of the enamel, appearing like white, opaque, "lifeless" areas, spots or lines, as illustrated in *figs. 1 and 2*. Covered by saliva and deposits they may be very hard to see, but during drying of the cleaned enamel their visibility

FIG. 2. "Pre-caries" (initial smooth-surface lesions). Left: second deciduous molar buccal surface. Right: deciduous 2nd incisor, mesial and labial surfaces.

increases in a manner suggestive of the development of a photograph. In the children studied it was found that these lesions occurred with the highest frequency in the permanent teeth. Since the second type of enamel lesion, referred to above, probably represents the very first clinically visible sign of tooth destruction, it may be named "pre-caries".

COMMENTS

The question may be raised as to whether the superficial enamel lesions just described should be included in a caries record. It is obvious that they can not be classified as cavities, a term which in general is used synonymously with dental caries. However, as in any other disease, it is highly desirable to recognize the first clinical manifestations. It is important from a practical viewpoint because

[2] Faults in the surface texture also manifest themselves as roughnesses in the enamel, but do not attain the whitish, dull appearance when dried as do the post-eruptive decalcification areas.

these initial smooth-surface lesions offer possibilities for prophylactic treatment, i.e., by the use of Howe's ammoniacal silver nitrate (4) or the remineralization treatment suggested by Andresen (5). Furthermore, in clinical studies of the control of dental caries, the progress of these lesions serves as an indication of the activity of the destruction. Although the lesions described as "pre-caries" do not necessarily develop into cavities, rapid progress has been observed in children (6) as well as in adults (7) under certain conditions. A survey made in this infirmary (8) brought out that more than 70 per cent of the children showed evidence of such lesions on the buccal and labial smooth surfaces of the teeth. Hence, any dental record would be incomplete unless it includes these easily overlooked manifestations of tooth destruction.

SUMMARY

1. An examination of the caries frequency in 32 children has been made to determine (1) to what extent the clinical findings vary when different diagnostic procedures are employed, and (2) what type of lesions are most liable to be overlooked. 2. It was found that nearly all carious cavities and pit and fissure lesions could be disclosed by mirror and explorer without additional diagnostic aids, except for smaller approximal cavities, discovered by radiograph. 3. In addition, another 20 per cent of the total number of lesions would have been overlooked if the teeth had not been cleaned and dried. Those were initial stages of smooth-surface lesions. The term "pre-caries" has been suggested for the first manifestations of these lesions, and their practical and theoretical importance has been emphasized.

The author wishes to offer his sincere thanks to Dr. Percy R. Howe, Director of the Forsyth Dental Infirmary, for the opportunity to carry out this study, and to Dr. Basil G. Bibby for his helpful suggestions during the preparation of this manuscript.

REFERENCES TO LITERATURE

(1) GYTHFELDT, T., *Norske Tannlaegeforenings Tidende* **48:** 243, 1938
(2) BOEDECKER, C. F., *D. Survey* **7:** 59, 1931
(3) BOEDECKER, C. F., *J. A. D. A.* **26:** 1453, 1939
(4) HOWE, P. R., *D. Cosmos* **59:** 891, 1917
(5) ANDRESEN, V., *The Physiological and Artificial Mineralization of the Enamel* (Oslo, Einar Dancke: 1926)
(6) SOGNNAES, R. F., AND WHITE, R. L., *Am. J. Dis. Children* (in press)
(7) SOGNNAES, R. F., *Munnpleien* **23:** 13, 1938
(8) SOGNNAES, R. F., Unpublished data.

VARIATION IN TOOTH POSITION FOLLOWING EXTRACTION OF FIRST MOLARS IN RELATION TO INCIDENCE AND DISTRIBUTION OF DENTAL CARIES[1]

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DISTRIBUTION OF EXTRACTED FIRST PERMANENT MOLAR BY JAW QUADRANTS

In the course of dental examinations of over 8,000 vocational school pupils during a period of 3 years, 3 outstanding conditions were noted: (1) The frequent loss of first permanent molars; (2) changes in positions of first and second premolars and second molar in the jaw quadrants from which the first permanent molars had been extracted and (3) a high incidence of "exteeth" (Teeth showing physical abnormalities acquired since their eruption; e.g., caries, fillings, redecay, roots remaining, extractions, etc.).

Full maxillary and mandibular dental impressions were taken and casts made of 500 boys and girls, 15 to 19 years of age, who were found to have had from 1 to 4 first molar teeth extracted (Table I). Additional groups of boys and girls in the same age range who had not lost any of the first molars were also studied for purposes of control and comparison. Altogether, 1940 boys and girls were studied, embracing the various ethnic, economic, social and intellectual components of the school population of Greater New York.

Table I shows the number and percentage of children by age groups, from 15 to 19 years, with 1 to all 4 M1's extracted; the total number of cases in each age group; the total number in each category having one, two, three or four M1's extracted per case; the total number of cases studied; the total number of M1's extracted, and the average number of M1's extracted per case in each age group, as well as the average number of M1's extracted per case for the group of 500.

[1] Presented at the New York Section of the International Association for Dental Research, June 1, 1939.

Table I shows that there is little differentiation with regard to age in the number of M1's extracted.

The distribution of the extracted first molars in the 4 jaw quadrants of the 500 cases examined was as follows: There were 14 per cent extracted in the maxillary right; 16 per cent in the maxillary left; 33 per cent in the mandibular right, and 37 per cent in the mandibular left quadrant of the jaws or 30 per cent in the maxilla to 70 per cent in the mandible, with the left side of each jaw exceeding the right (1).

TABLE I

Number and percentage of molars extracted in 500 children

AGES	MOLARS LOST								TOTAL NO. CHILDREN	TOTAL MOLARS LOST	NO. MOLARS PER CHILD
	One Molar		Two Molars		Three Molars		Four Molars				
	No.	Per Cent	No.	Per Cent	No.	Per Cent	No.	Per Cent			
15	31	43.7	29	40.8	7	9.8	4	5.7	71	126	1.77
16	84	42.0	77	38.5	30	15.0	9	4.5	200	364	1.82
17	53	36.0	53	36.0	31	21.1	10	6.8	147	292	1.98
18	23	39.7	18	31.0	14	24.2	3	5.1	58	113	1.95
19	9	37.5	7	29.2	5	20.8	3	12.5	24	50	2.02
Total	200		184		87		29		500	945	1.88

TYPES OF MOVEMENT OF FIRST AND SECOND PREMOLARS AND SECOND MOLAR AFTER EXTRACTION OF FIRST MOLAR

The first and second premolars and second molars in the jaw quadrants from which the first molars had been extracted showed change of position usually attended by rotation and inclination. The following symbols were used to designate the various combinations of movement studied:

0—0—0 = no movement of premolars or second molar
0—D—0 = movement of second premolar distally
D—D—0 = movement of both premolars distally
0—0—M = movement of second molar mesially
0—D—M = movement of second premolar distally and second molar mesially
D—D—M = movement of first and second premolars distally and second molar mesially

Table II shows the number and percentage of quadrants in each of the 6 designated positions distributed according to the various intervals between the time when the respective M1's were extracted and the examination for the purpose of this study was made. The total number and percentage of jaw quadrants is also shown for each of the designated positions.

TABLE II

Number and percentage of quadrants in each position distributed according to the intervals from 3 months to 12 years, between the extraction of the first molar and the taking of the impression.

POSITIONS		INT.'S													TOTAL QUADRANTS	
		3 mos.	6 mos.	1 yr.	2 yrs.	3 yrs.	4 yrs.	5 yrs.	6 yrs.	7 yrs.	8 yrs.	9 yrs.	10 yrs.	11 yrs.	12 yrs.	No.	per cent
PM1—PM2—M2	No.	7	4	3												14	1.5
0 0 0	%	50	28.5	21.5													
PM1—PM2—M2	No.	1	2	2		1										6	.6
0 D 0	%	16.6	33.3	33.3		16.6											
PM1—PM2—M2	No.	2	2	13	14	11	1	4	4		3			1		55	5.8
D D 0	%	3.6	3.6	23.6	25.4	20.0	1.8	7.2	7.2		5.4			1.8			
PM1—PM2—M2	No.	11	7	31	22	18	8	12	6	5	7				1	128	13.6
0 0 M	%	10.1	5.4	24.1	17.1	14.0	6.0	9.0	4.6	3.9	5.4				.75		
PM1—PM2—M2	No.	3	8	17	17	17	11	11	4	7	5	1				101	10.7
0 D M	%	2.9	7.9	16.8	16.8	16.8	10.8	10.8	3.8	6.9	4.9	.9					
PM1—PM2—M2	No.	4	16	84	92	82	77	92	62	51	49	11	16		1	637	67.6
D D M	%	.6	2.5	13.2	14.4	12.9	12.1	14.5	9.7	9.0	4.7	1.8	2.5		.2		
Total																941	99.8

Fig. 1 shows a comparison of a graph of the distribution into the designated positions of all jaw quadrants without regard to the time interval following the extraction of the M1's with a graph of the distribution at a time interval of 3 years. We can see here that the interval is no longer a factor in determining the type of tooth movement.

CLOSURE OF SPACE AFTER EXTRACTION OF FIRST MOLARS

When we measured the amount of space remaining at various intervals after the extraction of the M1's, we found that although the

space remaining tended to grow smaller as the interval after extraction increased, the rate of closure was not at all uniform in all instances. Furthermore, the space may never close completely, as in some cases. However, the median weighted average amount of space remaining becomes gradually smaller as the interval after extraction increases.

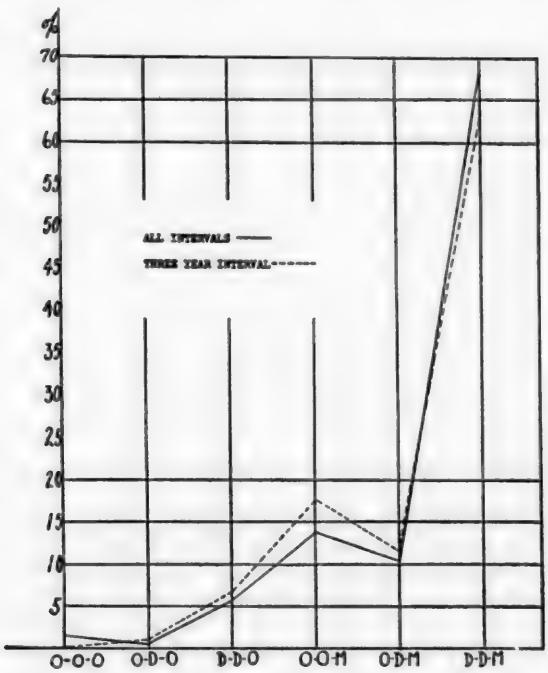


FIG. 1. Percentage distribution into designated positions of PM1—PM2—M2 (941 jaw quadrants in 500 children), including *all* intervals after extraction of M1, compared with a group at an interval of three years *only* after extraction of M1.

Table III shows the weighted median amount of space remaining in the maxilla and mandible at various intervals after the extraction of M1. It can be seen that the space shows a gradual tendency to close and that it closes faster in the maxilla than in the mandible.

Table III shows also the weighted median amount of movement in millimeters of the PM2 and M2 in the maxilla and mandible respec-

tively, at different intervals after the extraction of M1. It can be seen here that the maxillary M2 shows by far the greater amount of movement. The mandibular M2 is next in the amount of movement. The mandibular PM2 shows a rate of movement closely related to that of the mandibular M2.. The maxillary PM2 shows less movement than any of the other teeth mentioned (2).

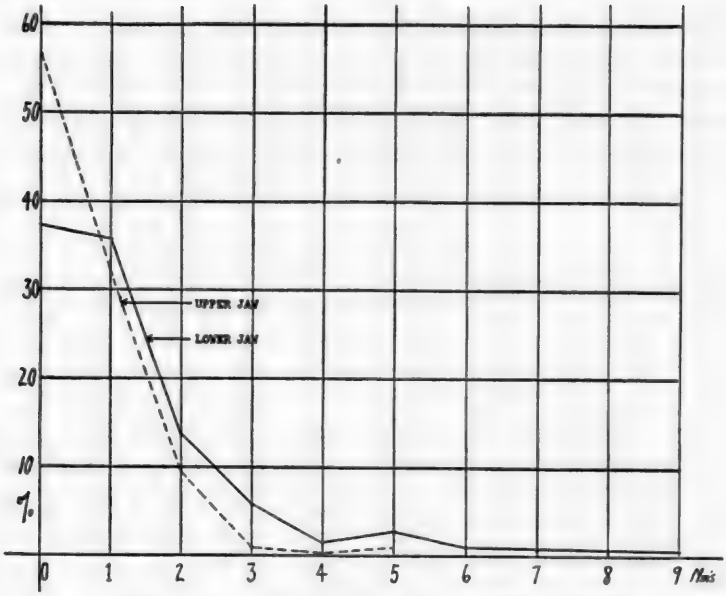
TABLE III

Weighted average amount of movement of PM2 and M2. Total weighted average amount of movement and weighted average amount of space remaining at different intervals after the extraction of M1

MAXILLA					MANDIBLE				
Time Int.	Weighted Aver. Movement		Total Wt'd Aver. Amt. Moved	Wt'd Aver. Space Remain.	Time Int.	Weighted Aver. Movement		Total Wt'd Aver. Amt. Moved	Wt'd Aver. Space Remain.
	PM2	M2				PM2	M2		
3 mos.	.5	1.3	1.8	8.8	3 mos.	.4	.9	1.3	9.4
6 mos.	1.4	2.1	3.5	7.3	6 mos.	1.0	1.2	2.2	8.5
1 yr.	2.1	3.5	5.6	4.7	1 yr.	1.9	2.4	4.3	6.3
2 yrs.	2.1	4.3	6.4	4.0	2 yrs.	2.6	2.8	5.4	5.0
3 yrs.	1.7	5.2	6.9	2.8	3 yrs.	2.7	3.6	6.3	4.1
4 yrs.	2.3	5.9	8.2	2.2	4 yrs.	3.4	3.6	7.0	3.4
5 yrs.	2.4	6.3	8.7	1.3	5 yrs.	3.8	4.4	8.2	2.2
6 yrs.	2.3	6.5	8.8	0.9	6 yrs.	4.3	4.4	8.7	1.9
7 yrs.	2.3	6.4	8.9	0.7	7 yrs.	3.8	5.2	9.0	1.8
8 yrs.	1.5	8.0	9.5	0.6	8 yrs.	4.2	5.0	9.2	1.5

COMPARISON IN RATE OF MOVEMENT OF PM1 AND PM2

From consideration of *fig. 2*, a graph, with its accompanying table we can see that PM1 and PM2 move at an equal rate more frequently in the maxilla than in the mandible, being even in 56.3 per cent of cases of the maxillary quadrants and in 37.8 per cent of mandibular quadrants. There is a constantly higher percentage of cases showing differences of 1 to 5 millimeters in the rate of movement between the first and second premolars in the mandible than in the maxilla. There are no cases in the maxilla where the difference in the amount of movement between PM1 and PM2 is greater than 5 millimeters. In the mandible we find cases showing as much as 9 millimeters difference in the amount of movement between PM1 and PM2.



Difference in amount of movement between PM1 and PM2
941 cases in 500 children
Median weighted averages in mm.'s

DIFFERENCE IN MM.'S	UPPER %	LOWER %
Even	56.3	37.4
1 mm.	32.1	35.8
2	9.4	14.3
3	1.	6.
4	.3	1.2
5	.7	2.7
6	0	.9
7	0	.9
8	0	.45
9	0	.15

FIG. 2. Percentage of cases and amount of difference (in millimeters) in rate of movement between PM1 and PM2 in upper and lower jaws after extraction of M1's in 500 children.

Table IV shows the weighted median amount of difference in millimeters between the amount of movement of PM1 and PM2 for the maxilla and mandible respectively, at different time-intervals

between the extraction of the M1's and the examination for the present study.

TABLE IV

Difference between PM1 and PM2 in mm. at different intervals after extraction of 941 M1's in 500 children

	UPPER	LOWER
3 mos.	0 Mm.	.23 Mm.
6 mos.	.7	.52
1 year	.8	.85
2 years	.6	.98
3 years	.7	1.3
4 years	.6	1.4
5 years	.5	1.3
6 years	.5	1.9
7 years	.5	1.4
8 years	.47	1.8

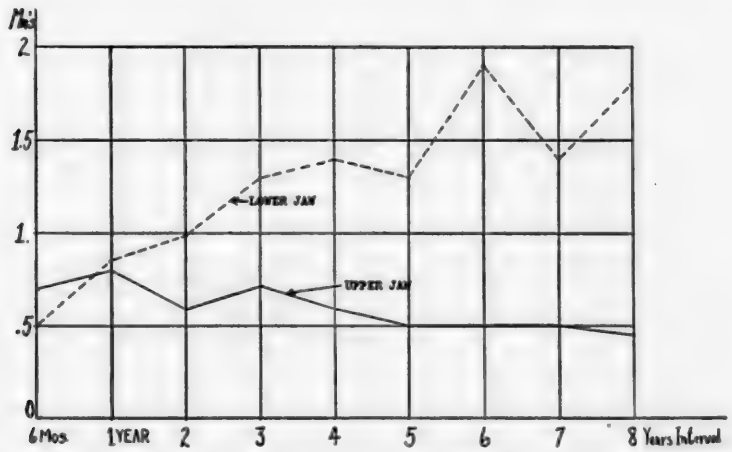


FIG. 3. Average (weighted) amount of space in millimeters between PM1 and PM2 at different intervals after extraction of 941 M1's in 500 children.

Fig. 3 shows a graph based on the data in Table IV. It is evident here that, while weighted median differences in the amount of movement between PM1 and PM2 in the maxilla reach a maximum of 0.8 mm. at the end of 1 year and show a tendency to decrease thereafter,

in the mandible, the tendency is for the difference in the amount of movement between PM1 and PM2 to show a gradual increase with the increase of the interval after extraction of M1.

FIRST MOLAR EXTRACTION AND CARIES INCIDENCE

A group of 940 boys and girls 15 to 19 years of age who had not lost any first molar teeth was examined. A record was made of the number of teeth in each mouth that were no longer in the original physical state as at the time of eruption; i.e. those having fillings; those that had been extracted, and those showing redecay or presenting any other clinical evidence of not being in the physical state that teeth usually are at the time of eruption. For the sake of convenience,

TABLE V

Comparison of exteeth when M1's are missing or when filled or carious

M1's per child	M1's missing	M1's filled or carious	Exteeth per child	
			M1 Missing	M1 F or C
0		150		1.4
1	200	190	9.2	3.2
2	184	200	10.3	5.6
3	87	200	11.0	7.8
4	29	200	14.0	9.4

Total average (M1 M 10.2)
(M1 F or C 5.7)

these teeth were designated as "exteeth". Malposed, hypoplastic or mottled enamel and other congenital abnormalities were not considered "exteeth". "Exteeth" do not include teeth with obviously pre-eruptive defects (3).

Table V shows a comparison of a group of boys and girls 15 to 19 years of age with all M1's free from caries or with 1 to all 4 M1's filled or carious with a second group in the same age range which had lost 1 to all 4 M1's. The average number of "exteeth" per case is shown for those with all M1's free from caries or with 1 to all 4 of these teeth filled or carious, compared with the average number of "exteeth" per case in the group with 1 to 4 M1's extracted. The total average number of "exteeth" for those with M1's filled is 5.7 for those with M1's extracted it is 10.2 per case. It must be kept in mind, however,

that this correlation does not take into consideration the state of the remaining M1's in the group with extracted M1's.

Table VI shows the average number of "exteeth" per case in the mouth as a whole, excluding "exteeth" in the M1's themselves, in the various age groups with 0 to 4 M1's filled or carious. This table

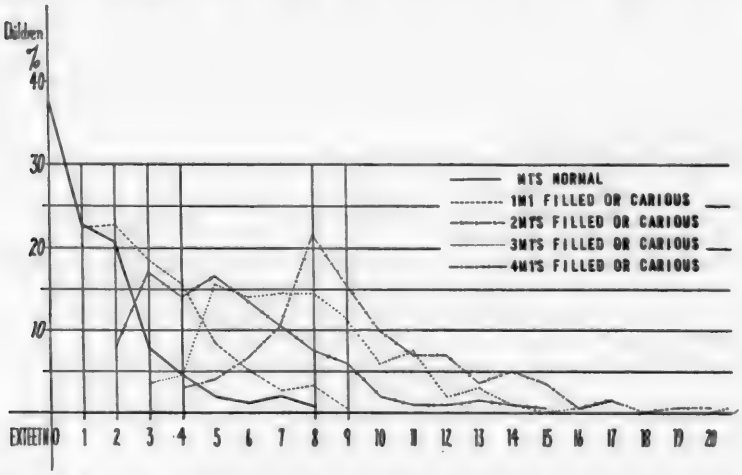


FIG. 4. Percentage of children with exteeth in 0 to 4 M1's related to total number of exteeth per mouth.

TABLE VI

Average number of exteeth per child (15–19 years) excluding M1's related to the number of M1's per child filled or carious

AGE	0	1	2	3	4
15	1.8	2.	2.7	4.7	5.8
16	1.3	2.	3.4	4.0	5.1
17	1.4	2.3	3.5	5.1	5.3
18	1.8	2.5	4.0	5.8	5.8
19	.91	2.0	4.9	7.1	7.6

does not include cases with M1's extracted. There is a rise shown in the caries incidence in the mouth as a whole with the increase in the actual number of "exteeth" in the M1's themselves.

Fig. 4 shows frequency graphs for each group with 0 to 4 M1's filled or carious, subdivided into categories from 0 to 21 "exteeth" per case.

The peak for those with all M1's in normal condition is found in the subdivision with 0 "exteeth" per case; the peaks for those with 1 M1 filled or carious is found in the subdivisions with 1 to 2 "exteeth"; the peaks for those with 2 M1's filled or carious is found in the subdivisions with 3 to 5 "exteeth"; the peak for 3 M1's filled or carious is found in the subdivisions with 5 to 8 "exteeth" and the peaks for those with all 4 M1's filled or carious is from 7 to 10 "exteeth" per case.

Table VII shows the average number of "exteeth" in a group of boys and girls 15 to 19 years of age with 0 to 4 M1's filled or carious, compared with respective similar age groups who had 1 to 4 M1's extracted and whose remaining M1's were either free from caries or

TABLE VII

Average number of exteeth per mouth excluding M1's related to actual number of M1's extracted, filled or carious per mouth and compared with average number of exteeth per mouth when M1's are filled or carious only

NO. M1'S FILLED OR CARIOUS	NO. M1'S EXTRACTED				
	0	1	2	3	4
0	1.4	3.1	3.5	6.9	10.6
1	2.2	3.0	5.0	7.3	
2	3.6	4.0	7.2		
3	4.8	6.8			
4	5.4				

showed a progressive increase of fillings or caries. In Table VII the "exteeth" in the M1's themselves have been omitted so that the averages would not be influenced by the "exteeth" in the M1's themselves but would show the incidence of "exteeth" in the rest of the mouth in relation to the number of "exteeth" in the M1's. We find here again that the average number of "exteeth" in the mouth as a whole rises with the increase in the actual number of filled or carious M1's and that the increase is intensified with the increase in the actual number of M1's extracted.

Fig. 5 is a bar graph which shows the average number of "exteeth" per case in a group with 0 to 4 M1's filled or carious compared with another group with 1 to all 4 M1's extracted. This graph indicated a tendency for those cases with 0 to 4 M1's extracted but whose remaining M1's are in perfect condition to show a higher average

number of "exteeth" over those with all of their M1's present but with a corresponding number of them filled or carious. In other words, a boy or girl with 1, 2, 3 or 4 M1's filled or carious shows a lesser average number of "exteeth" for the mouth as a whole, than one with a similar number of M1's extracted, when the rest of the M1's remaining in the mouth are in a normal condition (4).

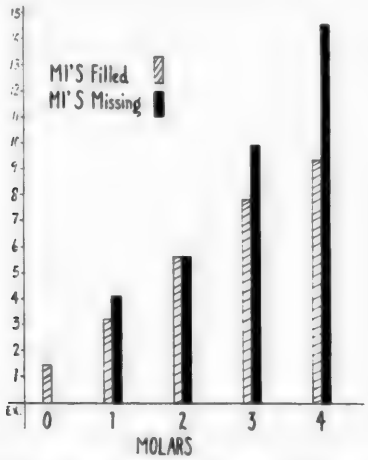


FIG. 5. Average weighted number of exteeth per child related to actual number of M1's filled or carious compared with actual number of M1's extracted.

EXTRACTION OF FIRST MOLARS IN RELATION TO DISTRIBUTION OF CARIES

Table VIII shows a comparison by jaw quadrants of 500 boys and girls, 15 to 19 years of age, with 1 to 4 M1's extracted with a similar age group of 500 with all M1's present. It is to be seen here that where the M1's are present there is a comparatively greater degree of correlation in the number and percentage of "exteeth" in each quadrant than when the M1's are extracted. However, there is a tendency shown for the maxillary teeth to have an increased number of "exteeth" in mouths with M1's extracted, in spite of the fact that, as previously stated, there were more than twice as many M1's extracted in the mandible than in the maxilla.

Table IX shows 500 cases with M1's extracted and 500 cases with

M1's present, compared according to the following respective frequencies: (1) The number of instances in which the "exteeth" in the maxillary jaw quadrants exceeded those in the opposing mandibular quadrants; (2) the number of instances in which the "exteeth" in

TABLE VIII

Comparison of exteeth by jaw quadrants in 500 children with M1 present and 500 children with M1 extracted. Ages 15 to 19 years

LOCATION	M1 PRESENT		M1 EXTRACTED	
	No.	%	No.	%
U. R.	949	25.2	1400	27.2
U. L.	990	26.2	1437	27.8
L. R.	921	24.4	1159	22.5
L. L.	915	24.2	1151	22.5
Totals	3775	100.0	5147	100.0

TABLE IX

Distribution of exteeth in opposing upper and lower jaw quadrants after extraction of 941 M1's in 500 children compared with distribution of exteeth in 500 children with M1's present

	MORE EXTEETH IN UPPER		MORE EXTEETH IN LOWER		EVENLY DISTRIBUTED UPPER AND LOWER EXTEETH		TOTAL	
	No.	%	No.	%	No.	%	No.	%
Upper M1 extracted	60	46.5	23	17.8	46	35.6	129	12.9
Lower M1 extracted	239	47.3	121	23.9	145	28.7	505	50.5
Upper and lower M1 extracted	97	58.0	23	13.8	47	28.1	167	16.7
Upper and lower M1 present	75	37.7	48	24.1	76	38.1	199	19.9
Totals	471	47.1	215	21.5	314	31.4	1000	100.0
500 children with M1's present	330	33.0	333	33.3	337	33.7		

the mandibular jaw quadrants exceeded those in the opposing maxillary quadrants and (3) the number of cases in which the "exteeth" were evenly distributed in the opposing maxillary and mandibular jaw quadrants. The foregoing observations were made on jaw quadrants falling into the following categories: (a) when the maxillary M1 was

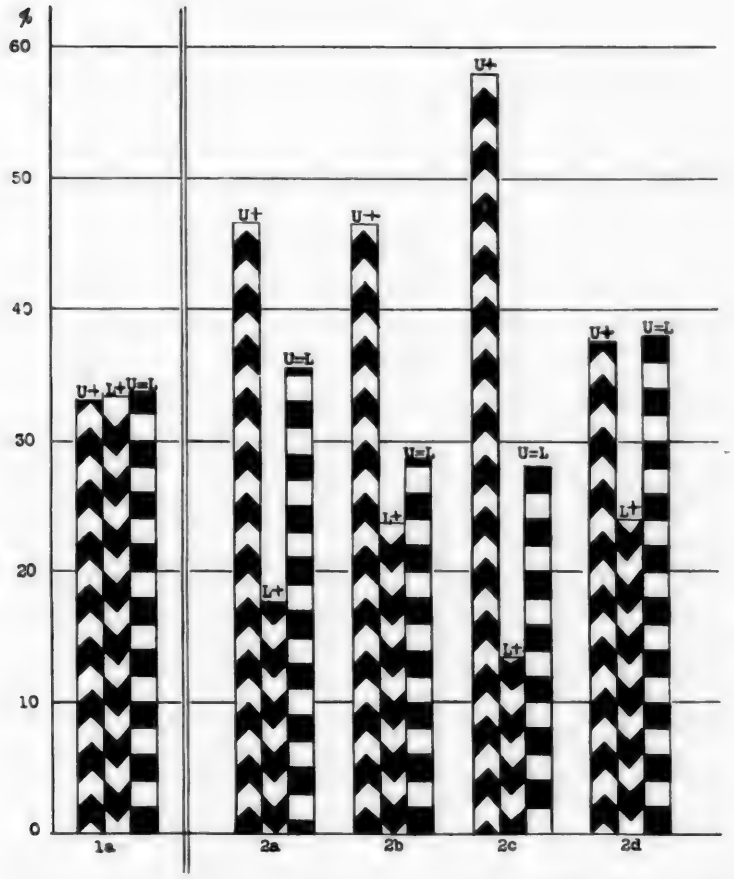


FIG. 6. Distribution of exteeth in opposing jaw quadrants in 500 children with M1's present compared with distribution of exteeth in opposing jaw quadrants of 500 children after extraction of M1's.

U+ = More exteeth in maxillary quadrant than in opposing mandibular quadrant.
L+ = More exteeth in mandibular quadrant than in maxillary quadrant.
U=L = Number of exteeth in maxillary quadrant equals that in opposing mandibular quadrant.

1a. 500 children with M1's present.

2a. Children with M1's extracted in maxillary quadrant and present in opposing mandibular quadrant.

2b. Children with M1's extracted in mandibular quadrant and present in opposing maxillary quadrant.

2c. Children with both maxillary and opposing mandibular M1's extracted.

2d. Children with M1's present in both opposing jaws of one side of face and one or both M1's extracted on opposite side of face.

extracted; (b) when the mandibular M1 was extracted; (c) when both the maxillary and mandibular M1's in the opposing quadrants were extracted and (d) where both the maxillary and mandibular M1's in the opposing quadrants on one side were present but where one or both M1's were missing on the opposite side of the jaws. An analysis of the distribution of "exteeth" in 500 cases with all first molars present is also shown.

Fig. 6 is a bar graph based on the data presented in Table IX. We can see here that, when all of the M1's are present the frequency of maxillary quadrants showing more "exteeth" than the mandibular and the frequency of mandibular quadrants showing more "exteeth" than the maxillary, as well as the frequency with which the opposing upper and lower quadrants show an equal number of "exteeth" are practically the same. However, in the bars representing quadrants with maxillary M1's extracted we find an increase in the frequency of quadrants showing more "exteeth" in the maxilla and a decrease in the mandible. In the bars representing the quadrants with mandibular M1's extracted we again find an increase in the frequency showing more "exteeth" in the maxilla and a corresponding decrease of quadrants showing more "exteeth" in the mandible. In the columns representing the quadrants with maxillary and mandibular M1's extracted we again find a marked increase in the frequency of quadrants showing more "exteeth" in the maxilla. In the columns representing opposing quadrants with M1's present but with 1 or both M1's missing on the opposite side of the mouth, we again find the frequency showing more "exteeth" in the maxilla to be greater than that in the mandible.

It appears, therefore, that where all M1's are present there is an equal distribution of frequencies showing more "exteeth" in the maxilla, the mandible and equal in both jaws. To the contrary, in mouths from which M1's have been extracted, regardless whether the extraction is in the maxilla or mandible, there is always an increase in the frequency of quadrants showing more "exteeth" in the maxilla.

RESULTS

1. In examinations of 500 boys and girls 15 to 19 years of age, an average of 1.88 M1's were found extracted per case, in the following

order of increasing frequency; maxillary right, maxillary left, mandibular right and mandibular left; the last being the most frequently extracted M1. There were 70 per cent of the total number of M1's extracted in the mandible and 30 per cent in the maxilla. The maxillary and mandibular left quadrants respectively showing an increase over the corresponding quadrants on the right side in the number of M1's extracted.

2. A study of the change of positions of the PM1, PM2 and M2 in the 941 quadrants from which the M1's had been extracted shows 67.6 per cent with distal movement of PM1 and PM2 and mesial movement of M2 (D—D—M); distal movement of the PM's only (D—D—0) was found in 5.8 per cent while 13.6 per cent showed movement of the M2 mesially only (0—0—M). The latter is the type of movement that those who extract first molars as an orthodontic aid expect to find in all cases. Furthermore, the D—D—M position only was found to prevail after a time interval of 8 years, with but few remaining in the other categories after 3 years. All other positions than D—D—M tended to disappear as the interval of time since the extraction increased. After 3 years the time interval is no longer a deciding factor in determining the type of movement of PM1, PM2 and M2 in quadrants from which the M1's have been extracted.

3. The space remaining after the extraction of M1 decreased in mesio-distal diameter as the time-interval following the extraction increased. The closure of the space is not uniform in all cases, and was found to be affected by environment, habit and other conditions. The space in the maxilla closes at a faster rate than that in the mandible. The maxillary M2 shows the fastest rate and the greatest amount of movement. The maxillary PM2 showed the least amount of movement. The respective amount of movement of the mandibular PM2 and M2 is practically equal in rate.

4. The rate of distal drifting of PM1 was found equal to PM2 in the maxilla in 56.3 per cent of cases while in the mandible the rate was equal in 37.4 per cent of cases. The difference in the weighted average amount of drift in millimeters between PM1 and PM2 was found to be higher in the mandible than in the maxilla. These differences persisted for longer intervals after the loss of M1's in the mandible than in the maxilla.

5. The average number of "exteeth"[2] in the mouth as a whole increases with the increase in the number of "exteeth" in the M1's themselves. Cases with M1's extracted average a higher incidence of "exteeth" for the mouth as a whole when compared with similar age groups having an equal number of M1's filled or carious but remaining in the mouth. The incidence of "exteeth" in the mouth as a whole is intensified with each additional M1 extracted.

6. "Exteeth" are more evenly distributed among the jaw quadrants when the M1's are retained in the mouth. In boys and girls with 1 to all 4 M1's extracted, the average number of "exteeth" in the mouth as a whole is increased and is higher in the maxillary teeth than in the mandibular.

7. In a group of 500 boys and girls 15 to 19 years there is an equal distribution of cases in which the maxillary quadrants show more "exteeth" than the mandibular quadrants or conversely, where the mandibular quadrants show more "exteeth" than the maxillary quadrants as well as in the frequency showing the number of "exteeth" in the maxilla to equal those in the mandible. However, in a group of 500 boys and girls, 15 to 19 years of age with 1 to 4 M1's extracted, the frequency of "exteeth" in the maxillary teeth is found to be higher than in the mandibular teeth, regardless of the jaw from which the M1's are extracted.

SUMMARY

According to the data presented, the boys and girls examined at 15 to 19 years of age show an increase of "exteeth" in the maxilla over the mandible when they have 1 to all 4 M1's extracted, regardless as to which jaw the extraction occurred. The rate of distal drift of PM1 and PM2 is more frequently even in the maxilla than in the mandible. The difference in the rate of movement between PM1 and PM2 in the mandible persists over a longer interval of time after the extraction of M1 than in the maxilla. The distal drift of the maxillary PM1 and PM2, as is indicated in the foregoing, is accompanied by loss of contact points without apparently sufficient separation of the respective teeth occurring to allow food debris to be freely

[2] Exteeth are teeth not in the original physical state as at the time of their eruption.

removed from between these teeth. In the mandible the difference in the rate of drift between PM1 and PM2 is greater than in the maxilla and food may be expelled more readily from between these teeth. Furthermore, in cases where the mandibular M1 has been extracted, there is an elevation or continuous eruption of the opposing teeth and consequent loss of contact point in the maxillary teeth. It is reasonable to assume, therefore, that the difference in the rate of drifting between PM1 and PM2 in the respective jaws is responsible for the increase in exteeth distribution in the maxilla in mouths from which 1 to 4 M1's have been extracted.

On the basis of the data herein presented, it appears that when the dentist fills first molar teeth, he does not simply perform a mechanical job which preserves these important teeth for use in chewing, but he also helps to maintain the occlusal relationship of all teeth in the mouth and lowers the incidence of caries in the mouth as a whole.

REFERENCES TO LITERATURE

1. SALZMANN, J. A., *Internat. J. Ortho.* 23: 662, 1937.
2. *Idem, J. A. D. A.* 25: 892, 1938.
3. *Idem, J. D. Soc. N. Y. State* 8: 1938.
4. *Idem, J. A. D. A.* 26: 1991, 1939.

STUDIES ON THE ACID SOLUBILITY OF HUMAN ENAMEL[1]

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Experiments designed to determine the effect of acid on tooth enamel were carried out by a number of investigators prior to the publication of Miller's chemo-parasitic theory of dental caries. However, since these early studies will be included in a review (1, 2) of the theories of dental caries expressed before the work of Miller, they will not be considered here.

Mummery (3), Pickerell (4), Dobbs (5), Thurlow (6), Hoff (7), Enright (8), Mizuma (9) and McIntosh (10) all have reported variations in the resistance to acid decalcification of enamel samples from various sources. Of these investigators Pickerell and Mizuma found the enamel of carious teeth to be more soluble in acid solution than enamel from non-carious teeth. Opposed to this are the findings of Klinger (11) and Kanthak (12) who were unable to observe any appreciable differences in the solubility of enamel obtained from the mouth of caries-free and caries-susceptible individuals. Only one report, that by Mizuma, has compared the relative solubility of permanent and deciduous enamel, deciduous enamel showing a greater acid solubility than permanent enamel. In a similar study Mizuma found young permanent enamel to be less soluble than the enamel of teeth taken from the mouths of older individuals. McIntosh, who investigated the same problem found just the opposite. Opposed to both of these reports are the findings of Kanthak who was unable to find any relationship between age and enamel solubility. Comparisons of the solubility of unerupted and erupted tooth enamel are meager, although both Pickerell and Dobbs have reported an apparent greater solubility in the case of the former.

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Unfortunately the great majority of authors have based their conclusions on a minimum of experimental data. This criticism should not be made of the investigators themselves, because in most cases these observations have been made incidental to the pursuit of a related problem. It was hoped that a more extensive investigation would clarify the following points: 1, the relative solubility of deciduous, young permanent, and old permanent enamels; 2, the relative solubility of the enamel of carious and non-carious teeth; 3, the relative solubility of the enamel of erupted and unerupted teeth.

METHODS AND MATERIALS

Enamel Samples. In an attempt to investigate the effect of age on enamel solubility, 3 groupings were established. They were: 1, deciduous teeth; 2, young permanent teeth taken from the mouths of people under 17 years of age; 3, permanent teeth taken from the mouths of adults over 25 years of age. The anatomical distribution of teeth within the 3 groups was approximately the same. To show the relationship between caries and enamel solubility enamel samples from 60 carious and a like number of non-carious teeth were tested. Unsupported enamel, previously cleansed from adherent carious dentin, was included as a portion of the carious samples. An attempt was made to keep the anatomical distribution of the teeth in each of the groups comparable. A total of 15 unerupted teeth were subject to solubility measurements. Two classes were recognized. They were: 1, root fully calcified; 2, root not fully calcified. The first group contained 7 teeth and the latter 8 teeth.

TABLE I

*The effect of particle size on enamel solubility**

MESH SIZE	NO. OF ANALYSES	AVERAGE INITIAL WEIGHT	AVERAGE WEIGHT DISSOLVED	AVERAGE DEVIATION OF THE AVERAGE
		mg.	mg.	mg.
100–200	4	50.0	19.65	0.25
200–300	4	50.0	21.95	0.30
300–400	4	50.0	23.0	0.05

* Young carious enamel dried in vacuo. Many of these teeth had been treated with silver nitrate (18).

Previous investigators (12, 14, 15) who have made solubility studies using powdered enamel, have employed enamel samples of undetermined particle size. To determine how this factor might affect the experimental results, enamel samples, prepared and purified by the "centrifugation-flotation" method of Manly (13) were separated into 3 sizes, 100–200 mesh, 200–300 mesh, and 300–400 mesh and tested for enamel solubility by the method to be described. Values of 19.65 mg., 21.95 mg. and 23.0 mg. respectively, were obtained. From these experimental results (see Table I) it is evident that the velocity of solution of enamel particles is proportional to their surface areas.

An interesting observation concerning the preparation of enamel samples was noted during the preliminary experiments. The first step in the preparation of enamel samples for Manly's separation technic consists of drying the teeth for a period of about one week at 110°C. It was found that enamel samples dried in vacuo at this temperature were markedly less soluble than samples subjected to the same heat in air. The explanation of this difference which in some cases was as great as 25 per cent is not known. However it was noted that vacuum drying caused an increased pigmentation in all enamel samples thus prepared.

Solubility measurements. Fifty milligram enamel samples, dried in air and size graded 100–200 mesh were weighed on a chainomatic analytical balance. The samples were transferred to 50 cc. round bottom centrifuge tubes. The test tubes containing the enamel were placed in a constant temperature bath at 38°C. Twenty cubic centimeters of

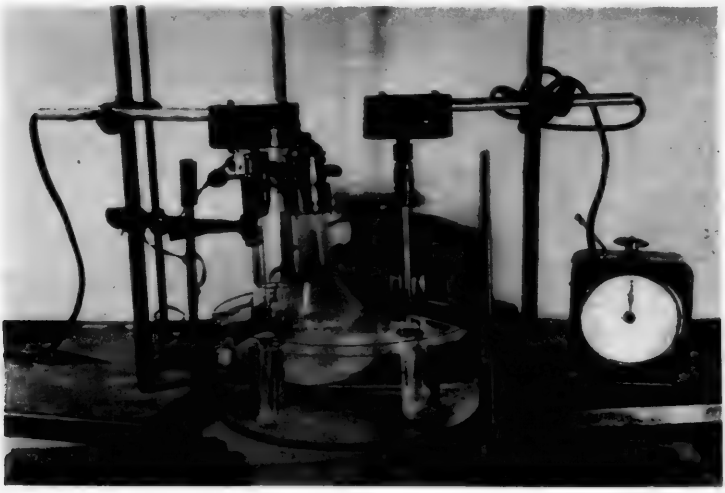

FIG. 1. ENAMEL SOLUBILITY MEASUREMENT APPARATUS

0.2 M acetic acid/sodium acetate solution with a pH of 4.0 and prepared according to the directions of Walpole (16) were added by pipette. Preliminary experiments demonstrated that buffer pairs of this concentration were capable of dissolving a sufficient amount of enamel powder in one hour to allow accurate observations concerning the relative solubility of the respective samples to be made. The mixture of enamel and buffer was agitated for one hour by an electrically driven glass stirring rod. The apparatus used may be seen in *fig. 1*. At the end of this period the stirring was discontinued and the test tubes were removed from the water bath, centrifuged, and a portion of the supernatant fluid decanted. To safeguard against any significant change in the pH of the buffered solution the hydrogen ion concentration of the decanted fluid was determined potentiometrically with a quinhydrone electrode. The remaining contents of the centrifuge tube were pressure filtered through a weighed filter crucible with a pore size 5–7 microns. After the enamel residue had been washed twice with distilled water, the

filter crucible was dried in an electric oven at 110°C. for 2 hours and cooled in a desiccator for the same period. Finally, the crucible was weighed and the weight loss of the original enamel sample calculated. All experiments were carried out in duplicate.

FINDINGS

The results may be seen in Table II. It was found that an average of 28.9 mg., 26.9 mg., and 26.0 mg. of the 50 mg. samples of deciduous, young and old enamel samples respectively were dissolved by 20 cc. of the buffer solution in 1 hour. Values of 25.2 mg. and 26.0 mg. for carious and non-carious enamel respectively were obtained under the same conditions. Similarly, values of 26.9 mg. for partially calcified

TABLE II

Solubility of 50 mg. samples of human enamels

SOURCE OF SAMPLE	NO. OF ANALYSES	AVERAGE WEIGHT DISSOLVED	AVERAGE DEVIATION OF THE AVERAGE
		mg.	*mg.*
Deciduous	6	28.9	0.70
Young	6	26.9	0.40
Old	6	26.0	0.20
Carious	6	25.2	0.30
Non-carious unerupted	6	26.0	0.30
Partially calcified, unerupted	7	26.9	0.20
Fully calcified	7	26.0	0.30

and 26.0 mg. of fully calcified resulted from the experiments on the unerupted tooth enamel.

DISCUSSION

Our finding that deciduous enamel was more soluble than permanent confirms the observation of Mizuma. A possible explanation of this phenomenon may be that deciduous teeth contain a greater percentage of magnesium and that the presence of magnesium salts increased the enamel decalcifying power of buffered solution (17). The close agreement between the solubility values of young and old permanent enamel substantiates the findings of Klinger and Kanthak. The slightly greater solubility of young enamel was observed by McIntosh and our results confirm his report.

The absence of a significant difference between the solubility of enamel from carious and non-carious teeth indicates that the resistance or susceptibility of a tooth to caries is not dependent on the relative acid solubility of the enamel. This conclusion is not at variance with the chemo-bacterial theory of dental caries which postulates that the carious process is initiated by the decalcification of the dental hard tissues. This finding merely points out that the enamel of all teeth would be decalcified by acid solutions at approximately the same rate, unless the acid solution was removed or neutralized by some external agency. The explanation of the slightly lowered solubility of carious enamel is not clear. One possibility is that the percentage of organic material in the carious samples is of a greater order than that of the non-carious samples because of the large amount of unsupported enamel included in the former. Enamel of this type may previously have been partially decalcified, resulting in the deposition of an increased percentage of organic material which would resist destruction by acids.

The results obtained from the solubility studies on unerupted teeth are not in agreement with the findings of Pickerell and Dobbs. However, this is not surprising since their conclusions were drawn from investigations conducted on the enamel of intact teeth and not powdered enamel. While their results may have more direct clinical significance, ours would give a clearer indication of there being a change in the substance of the enamel itself.

SUMMARY

A study of the acid solubility of different types of human enamel, using a gravimetric technique, revealed the following:

1. Deciduous enamel was more soluble than permanent enamel. No striking difference in the solubility of young and old permanent enamel was evident, although the experimental data suggested a decrease in enamel solubility with age.

2. Enamel from carious and non-carious teeth was not appreciably different in its resistance to solution by acids.

3. The values obtained for the enamel of unerupted teeth compared favorably with those already recorded for the enamel of erupted teeth.

REFERENCES TO LITERATURE

(1) VOLKER, J., *Acid Solubility Studies on Human Enamel*, Thesis, University of Rochester, Rochester, N. Y., 1939.
(2) Idem, to be published.
(3) MUMMERY, S., *Proc. Royal Soc. Med.* **3:** 71, 1909.
(4) PICKERELL, H., *Dental Caries and Oral Sepsis*, 3rd ed., (New York, Paul B. Hoeber, Inc., 1924) p. 112.
(5) DOBBS, E. C., *J. D. Res.* **12:** 581, 1932.
(6) THURLOW, S., AND BUNZELL, H., *D. Cosmos* **66:** 20, 1927.
(7) HOFF, P., AND KÖSZIG, F., *Zeit. fur Stomat.* **35:** 923, 1937.
(8) ENRIGHT, J., FRIESELL, H., AND TRESCHER, M., *J. D. Res.* **12:** 759, 1932.
(9) MIZUMA, M., *J. Biochem.* **25:** 261, 1937.
(10) MCINTOSH, J., JAMES, W., AND LAZARUS-BARLOW, P., *Brit. J. Exp. Path.* **3:** 138, 1923; **6:** 260, 1925.
(11) KLINGER, A., *D. Rec.* **58:** 303, 1938.
(12) KANTHAK, F., *J. D. Res.* **14:** 21, 1934.
(13) MANLY, R., *J. D. Res.* **18:** 133, 1939.
(14) KARSHAN, M., AND ROSEBURY, T., *J. D. Res.* **14:** 220, 1939.
(15) BENEDICT, H., AND KANTHAK, F., *J. D. Res.* **12:** 277, 1932.
(16) WALPOLE, G., *J. Chem. Soc.* **105:** 2501, 1914.
(17) FORBES, J., *J. D. Res.* **13:** 349, 1933.
(18) HILL, T. J., AND ARNOLD, F. A., *J. D. Res.* **16:** 23, 1937.

THE RADIOPAQUE SURFACE LAYER OF ENAMEL AND CARIES

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In 1934 Thewlis (1) made a radiographic study of ground sections of human teeth and described what he regarded as a hypercalcified outer layer. Hollander and Saper (2) considered this apparent radiopaque surface layer of the enamel as an artifact. In 1937 Thewlis (3) described microphotometer experiments indicating that the surface layer of the enamel is in fact hypercalcified. He found that there is "a steady increase in calcification from the amelodentinal junction outwards and the outer enamel layer is in many cases markedly hypercalcified."

Hollander and Saper (2) also noted that the radiopaque white line usually persisted on the surface of enamel over an area of decalcification corresponding to an early caries lesion. They concluded that this is also an artifact. Following this view the writer attached no significance to this line in grenz-ray studies of incipient caries lesions (4, 5, 6, 7). If the line is not an artifact, however, its significance needs investigation.

Pincus pointed out the paradox of decalcification occurring in enamel under an apparently intact outer layer. He suggested that the radiographic effect is due to bacterial invasion of the organic matrix of enamel rather than to acid attack (8).

To check up on the nature of the white line which persists on the surface of incipient caries lesions, the writer sent several grenz-ray negatives to Thewlis in England. He replied as follows:

"In my opinion the white line on the surface of the carious lesion is genuinely due to high x-ray absorption in all cases except one—no. 56, where it might be purely photographic. There are two main reasons why I do not think the effects are, in general, entirely photographic. They are:

1. The white line in these cases is not uniformly thick and its breadth is greater than that usually associated with the photographic effect.

2. The photographic effect depends for its existence on the presence of marked contrast between the two areas on the border of which it lies. In many of the present cases there is little or no contrast between the two sides."

Thewlis's point about the variable width of the white surface line should be emphasized. In some cases previously published (4, 5), striae of Retzius can be seen in this surface layer. Certainly the appearance of these striae in the wide white line cannot be dismissed as an artifact. The striae, disclosed by the acid, revealing the original calcification pattern, are clearly shown in *fig. 2*.

Thewlis also suggests that caries may have undermined the enamel, having started at some point which does not lie in the section examined. The writer has seen many sections which do not show a white line at the surface of the lesion (5, 7) and Hollander and Saper (2) have described lesions of this kind. Possibly caries started in the plane of such sections.

Incipient caries is best studied on the enamel of the smooth proximal surfaces of the teeth. In incipient caries of enamel an actual cavity has not yet been formed. Clinically, the enamel is often so hard that the defect cannot be detected with an instrument. Some lesions are white; others are brown or black. In some instances the beginning of decalcification cannot be detected with clinical (hard) x-ray apparatus.

In proximal caries, decay is apt to begin just on the gingival side of the contact point, and its occluso-gingival surface dimension is confined between that point and the free border of the gingiva. It is free to spread bucco-lingually as far as the sweep of food through the embrasures permits. These observations were made by G. V. Black years ago.

Penetration by acid on such a surface follows the direction of the enamel rods; its advance is cone-like, with the apex toward the dentin and the base toward the surface of the tooth. This method of penetration almost precludes the possibility of undermining of the enamel in this type of caries, which is entirely different in this respect from fissure caries.

As previously mentioned, many cases of incipient caries have been studied by the writer since 1933 (4, 5, 6, 7). Most of them had a radiopaque surface layer over the lesion. The possibility of undermining caries in specimens studied was nevertheless considered, and

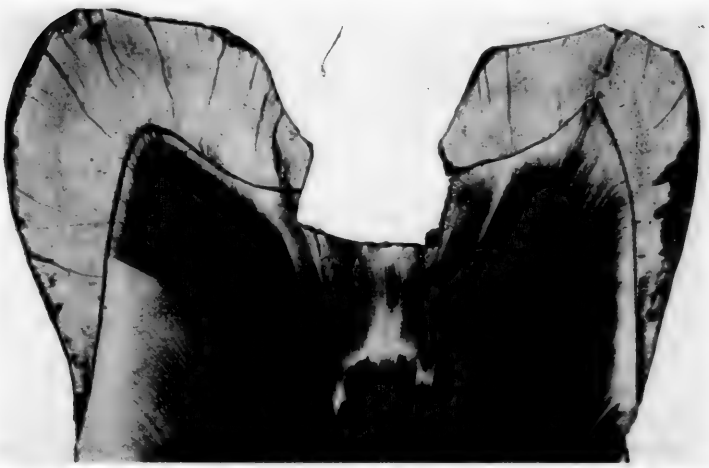

FIG. 1. Low power photomicrograph of human molar with incipient caries on mesial surface. Mesio-distal ground section cut near lingual surface through an incipient caries lesion. In this plane lesion has not penetrated far into enamel. Stripes of Retzius are disclosed presumably by penetration of acid. Note spread of caries toward gingival.

FIG. 2. Enlarged grenz-ray picture of part of ground section seen in *fig. 1*. The incipient caries lesion shows variation in width of surface radiopaque layer. Note variations in density of this layer and stripes of Retzius.

FIG. 3. Low power photomicrograph of another section of same tooth cut through center of incipient caries lesion. This is mesio-distal ground section cut nearer buccal surface. In this plane lesion has penetrated entire thickness of enamel.

FIG. 4. Enlarged grenz-ray picture of section shown in *fig. 3*. Decalcification extends through entire thickness of enamel. There is apparent radiopaque white line on surface of incipient caries lesion. Section was accidentally broken in several places. One crack goes right through center of lesion, yet there is no surface white line on edges of crack.

"*serial ground sections*" were made of other teeth. An upper molar with a mesial caries lesion was selected for study as a typical specimen. This tooth was cut into three mesio-distal ground sections through the lesion, one near the lingual surface, one through the center and one near the buccal surface. *Fig. 1* is the lingual section, seen under low power. In this section the lesion has not penetrated far into the enamel and the stripes of Retzius are clearly disclosed, presumably by

FIG. 5. Low power photomicrograph of another plane of same tooth. Mesio-distal ground section cut through incipient caries lesion near buccal surface. In this plane lesion is very superficial.

the penetration of acid. *Fig. 2* is an enlarged grenz-ray picture of the same section showing variation in density and width of the surface radiopaque layer. The stripes of Retzius are also indicated by variation in density.

Another section of the same tooth cut through the center of the incipient caries lesion (*fig. 3*) shows penetration of caries through the entire thickness of enamel. Similarly, a grenz-ray picture of this section (*fig. 4*) shows decalcification through the entire thickness of enamel. The surface radiopaque layer is nevertheless still present.

Here an accidental break occurred in the section directly through the center of the lesion. It is noteworthy that the two edges produced by the break did not show radiopaque white lines as would be expected if the effect were merely a surface artifact. This is further evidence that the white surface line is not an artifact in this radiograph. The third section cut from this same tooth (buccal plane) (*fig. 5*) showed only superficial penetration of the acid. The grenz-ray negative showed a surface layer over the decalcified area.

It seems evident that the persistence of a radiopaque white line on the surface of some incipient caries lesions cannot be dismissed as an artifact, but is to be considered a significant phenomenon. Three possible explanations may be offered. The first is that since hypercalcification of surface enamel evidently occurs normally, the removal of mineral matter in incipient caries may still leave more mineral matter at the surface than in the depth of the lesion. The second explanation is that caries may have undermined the enamel, having started at some point which does not lie in any of the sections examined. [This explanation must be considered because serial ground sections cannot include all planes of a lesion. There is evidence that decalcification is the essential lesion of incipient enamel caries (6). Pincus' suggestion of bacterial attack on organic enamel matrix (8) cannot explain the radiolucent areas seen in grenz-ray studies. This is due to loss of inorganic matter judging by artificial decalcification studies of enamel (9).] A third explanation is that the decalcified surface layer of enamel has been "remineralized" by saliva.

REFERENCES TO LITERATURE

(1) Thewlis, J., *Brit. D. J.* **57:** 457, 1934.
(2) Hollander, F., and Saper, E., *D. Cosmos* **77:** 1187, 1935.
(3) Thewlis, J., *Brit. D. J.* **62:** 303, 1937.
(4) Applebaum, E., Hollander, F., and Bodecker, C. F., *D. Cosmos* **75:** 1097, 1933.
(5) Applebaum, E., *D. Cosmos* **77:** 931, 1935.
(6) Applebaum, E., and Adam, T. H., *J. D. Res.* **17:** 95, 1938.
(7) Applebaum, E., *J. A. D. A.* **25:** 1223, 1938.
(8) Pincus, P., *Brit. D. J.* **63:** 511, 1937.
(9) Applebaum, E., *J. D. Res.* **17:** 181, 1938.

HISTOLOGICAL EVIDENCE OF THE HARMFUL EFFECT OF CEMENT UNDER ORTHODONTIC BANDS[1]

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Dental cements are used universally for securing bands during orthodontic treatment. These preparations contain a high content of phosphoric acid and may be a responsible factor in the decalcification of the enamel. An indication that this danger is real was noted in a recent report (1) which showed that dyes penetrated the enamel of the teeth of dogs only in those areas which were subjected to the action of oxyphosphate cement. It appeared evident that the action of the cement made the enamel abnormally permeable from without, so that the dye could penetrate the enamel, and, in certain areas, also the dentin. The normally impermeable surface of enamel is considered a barrier to dental caries and this property should be maintained to insure good dental health.

It has been observed clinically that small islands of decalcification, usually white in character, may be present on the enamel after the removal of anchor bands. They are particularly noticeable when the tooth is dry. Many clinicians removed bands periodically with the expectation that the saliva would not only arrest the etching of the enamel but would also recalcify it.

Etching of enamel has been produced experimentally through the action of weak acids, lactic acid being the most common. Mummery (2) and Bunting and Palmerlee (3) reported that the enamel was unevenly decalcified by acids. The work of Enright, Friesell and Trescher (4) verified these earlier reports. They demonstrated that decalcifications occurred with relatively weak acids and that the enamel had a variable resistance to the action of acid, i.e., certain isolated areas were more susceptible than others.

[1] Presented at the 17th General Meeting of the International Association for Dental Research, Cleveland, March 19, 1938. (*J. D. Res.* **18**: 264, 1939.)

Reports on the pH studies of dental cements indicate that they are sufficiently acid to decalcify the dental tissues. Crowell (5) in an extensive report on the chemistry of dental cement found that the highly acid liquid is probably neutralized by the mineral salts of the dentin. Paffenbarger et al. (6, 7) found that the liquids of oxyphosphate and silicate cements are similar. They contain about 50 per cent free phosphoric acid and have a pH of approximately 2. While the values of oxyphosphate cement are slightly higher, the only figures available are for silicate cement which vary from pH 2.8 to 3.7 fifteen minutes after mixing and from 4.5 to 5.6 twenty-four hours later. Most of the authors (5, 8, 9) discussing the harmful effects of cement draw the conclusion that calcium salts are removed from the dentin and that the acid has a deleterious effect on the pulp when cement is used as a filling material. It may readily be assumed that the calcium salts are also removed from the enamel.

There is a paucity of literature on the subject of decalcification of enamel after cementing orthodontic bands. While this report does not wish to establish that decalcification of the surface of enamel is a precarious lesion, it concurs with the opinion of Noyes (10) that clinically a great similarity exists between etched enamel and early stages of carious enamel. In a report upon the incidence of caries during orthodontic treatment, he attributed the decalcification to ill-fitting bands or to those cases in which the cement had broken and a leak occurred. Murless (11) advocated the use of silver nitrate as a protective measure to prevent the high incidence of caries after the removal of anchor bands.

EXPERIMENTAL TECHNIQUE

The assumption was made that dental cement etched the surface of enamel by chemical action. Twenty-one teeth, to which orthodontic bands had been cemented, were examined in this study. Unfortunately it was impossible to obtain teeth of young individuals at the usual age of orthodontic treatment. Eight patients of the school clinic who volunteered to permit banding of teeth, previously marked for extraction, ranged in age from 35 to 53 years. All teeth were examined by the Division of Oral Diagnosis and had neither stain nor incipient or arrested caries. The enamel was considered clinically and roentgenologically perfect. Precious metal bands were cemented on the teeth in the same manner as in orthodontic practice. Regular oxyphosphate cement, a silicate cement and gutta percha were used. They were permitted to remain in place for 7 days. The teeth were then extracted and ground sections made. All sections were photographed and the decalcification studied by grenz ray (12) examination.

RESULTS

Of 18 cases in which cement was used, 15 showed evidence of decalcification. The most marked was that of 2 cases where silicate cement was used. In 3 cases, where temporary stopping was used, no decalcification was noted. In an attempt to learn whether varnish is protective to the surface of enamel, the incisal halves of 4 teeth were

FIG. 1

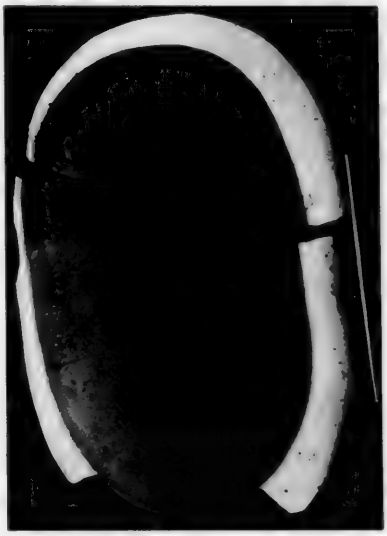

FIG. 2

FIG. 1. Photomicrograph of ground section maxillary premolar. Patient age 36. Band cemented (oxyphosphate cement) for 7 days. Decalcification on distal surface affecting broad area of narrow depth.

FIG. 2. Grenz ray of same section (*fig. 1*—photograph inverted). Note decalcification of same area. Degree of decalcification diminishes from periphery inward.

painted with stained varnish so as to be perceptible after sectioning. Preliminary observations indicate that the method is effective.

Fig. 1 is a photomicrograph of a ground section of a maxillary right second premolar of a 36 year old patient. A band had been secured on the tooth with oxyphosphate cement for 7 days. The distal surface shows a narrow band of decalcification indicated by a line running parallel to the surface of the section. A grenz ray of the same section (*fig. 2*) verifies this opinion, demonstrating a decrease in

decalcification as the dentino-enamel junction is approached. A similar result was shown by Bodecker (13) in a report on the decalcifying effect of commercial tooth cleaning preparations containing hydrochloric acid.

Some of the specimens examined simulated the white spots frequently found under orthodontic bands. *Fig. 3*, a ground section of

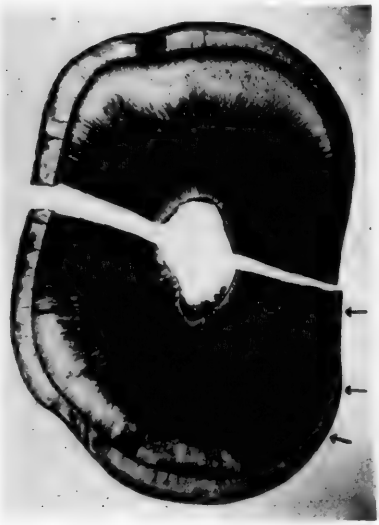

FIG. 3

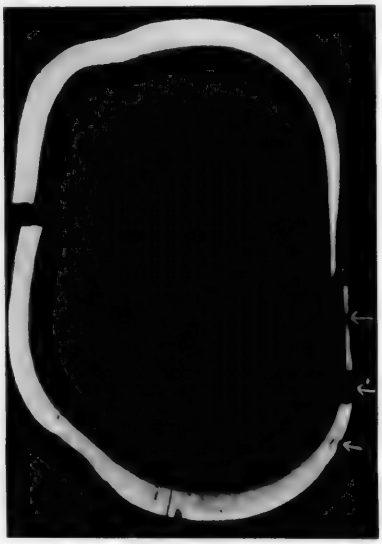

FIG. 4

FIG. 3. Photomicrograph of ground section maxillary premolar. Patient age 36. Band cemented (oxyphosphate cement) for 7 days. Arrow points to islands of decalcification.

FIG. 4. Grenz ray of same section (*fig. 3*). Arrows indicate islands of decalcification which are better defined than in *fig. 3*.

the mandibular right canine of a 36 year old patient, illustrates this point. This tooth was treated in the same manner as in the previous case. On the mesial surface there appears to be small isolated areas of decalcification. *Fig. 4*, a grenz ray of this section, shows 3 areas in which there has been a dissolution of the calcium salts of the enamel. There is a varying intensity of the lesion bearing out the contention of the in vitro experiment of Enright, Friesell and Trescher (4).

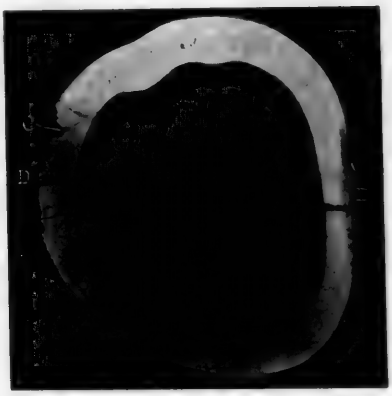

FIG. 5 FIG. 6

FIG. 5. Photomicrograph ground section of premolar. Patient age 35. Band cemented (silicate cement) for 7 days. C—carious enamel. D—decalcification. Note contrast in morphology beteen lesion of carious enamel and cement decalcification.

FIG. 6. Grenz ray of same section (*fig. 5*—photograph inverted). C—carious enamel. D—decalcification caused by dental cement.

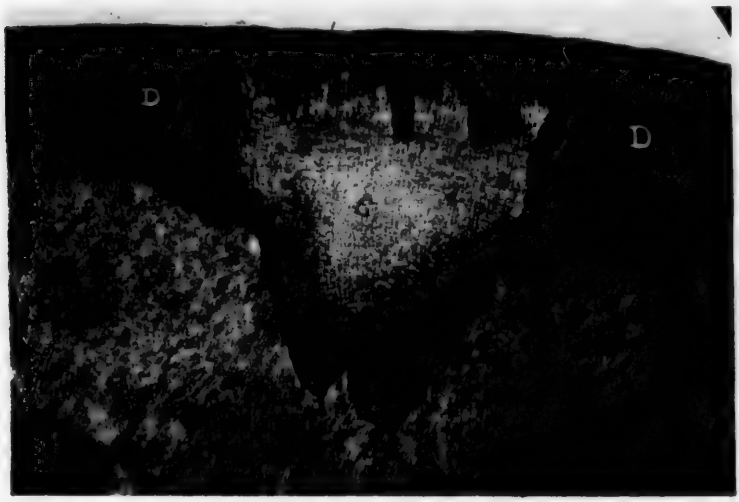

FIG. 7. Higher magnification of area C—*fig. 5*. D—decalcification produced by silicate cement in which enamel rods as well as cement substance was attacked. C—carious enamel. Note normal appearance of rods in carious area which is bordered by band of demarcation. In area of cement decalcification (D) rods not discernible. No band of demarcation present.

Fig. 5 is a photomicrograph of a section of the maxillary left second premolar of an individual 35 years of age. In this case the band was attached with a silicate cement for 7 days. Microscopic examination showed a small area of carious enamel at C. There is a general decalcification (D) on the mesial and distal surfaces quite dissimilar from the usual picture of carious enamel (C), which generally points towards the dentino-enamel junction in a cone-shaped manner. A grenz ray of the same section (*fig. 6*) shows wide areas of decalcification of rather uniform depth (D) whereas the carious area (C) takes the usual form of enamel caries pointing towards the dentino-enamel junction. The decalcified area on higher magnification (*fig. 7* D-D) shows that the enamel rods, as well as the rod sheaths were decalcified. The depth of the general decalcification (DD) caused by the cement is quite uniform, whereas at the carious area (C) the lesion has progressed in a funnel-shaped manner towards the dentino-enamel junction.

DISCUSSION

The fact that it was necessary to use teeth of adults rather than those of children makes these experiments even more striking. Adults are less susceptible to caries and decalcifications of enamel than children. This is probably due, according to Beust (14) and Bodecker (15), to the reduced permeability of adult teeth. The teeth of children are more permeable, hence the action of the phosphoric acid of the cement would probably result in more extensive decalcifications of the enamel. Criticism may be made that the decalcified areas shown in these illustrations were present before the teeth were banded. It is, of course, impossible to establish controls if the same teeth are to be used for experimental purposes. This matters little because histologically there is a marked difference between the initial lesion of carious enamel and the decalcification disclosed by this study. *Fig. 7* illustrates the difference between the two conditions. At C a carious area is shown bordered by the marked etching believed to be caused by cement. A typical band of demarcation is noted around the carious area which is lacking in the etched enamel. Most important in differentiating these two conditions is that in incipient caries, the enamel rods can still be defined whereas the cement decalcification

in this section alters this general picture. The enamel rods as well as the rod sheaths are decalcified making differentiation difficult.

The question may be raised: Why does not the phosphoric acid of dental cement attack the carious enamel? It appears paradoxical that the partially decalcified carious enamel should resist the acid and retain its original appearance. This observation, that carious enamel is more resistant to acid than normal enamel, was reported by Beust (16) who decalcified ground sections in 1 per cent lactic acid for 24 hours and found the carious enamel intact while the normal enamel was decalcified. This finding was later corroborated by Enright, Friesell and Trescher (4).

Applebaum, Hollander and Bodecker (12), in a grenz ray study of incipient caries shows the typical lesion to be cone-shaped with its base at the periphery of the enamel. The cement decalcification has no resemblance to this form; it appears as a narrow band of constant depth penetrating the surface of enamel (*figs. 2 and 6*) or in the complete dissolution of the inorganic salts of the enamel as illustrated in *fig. 4*. It appears as though the decalcifying action of cement is self-limiting. Two reasons may be advanced for this. Paffenbarger, Schoonover and Souder (7) showed that, upon setting, the cement was almost neutral and Karshan and Rosebury (17) showed that the dissolved calcium limits the decalcifying effect of an acid.

It is interesting to point out that the degree of decalcification of the carious enamel is greater than that produced by the dental cement. This was determined by varying the exposure of the grenz ray. A lesser exposure failed to show the cement decalcification.

It is evident from the results of grenz ray examination that cement decalcification occurs in localized areas of the enamel. While it is difficult to establish a definite reason for these vulnerable sites, it must be borne in mind that not all surfaces of enamel are equally attacked by dental caries. This destructive process, which undoubtedly begins with a dissolution of the calcific salts of enamel, does not affect the entire surface evenly, but attacks certain localized areas. Orban (18) in discussing the secondary enamel cuticle states that it is a keratinous substance resistant to both acids and alkalies. The loss of certain parts of the cuticle may be a factor in the unequal

etching of enamel under dental cement. On the other hand, we cannot disregard the natural resistance of the tooth to caries. Many reports have been made on this subject. It is the concensus of opinion among vitalists that an unknown element of resistance to caries and decalcification exists (15, 16). If the initial lesion of caries occurs with the decalcification of enamel at a vulnerable area, then, it may be assumed, that the highly acid liquid of dental cement may be responsible for similar irregularly distributed islands of decalcification of enamel.

Before concluding, emphasis must be placed on the following: 1. The teeth used for experimental purposes were not from children but from adults ranging in age from 35 to 53 years; a time when caries or decalcifications are more uncommon. In spite of this, decalcifications occurred under the cement. 2. The cement was not dissolved from under the bands, hence the decalcification could not be attributed to the decomposition of food. 3. The decalcification of the enamel produced by cements is not a true carious lesion, but there can be no doubt that such areas can be attacked more readily than the normal enamel because they form a zone of lowered resistance to the inroads of caries.

CONCLUSIONS

In vivo experiments on human teeth have presented evidence that dental cement decalcifies enamel. Unknown factors allow the acid to penetrate only certain of the areas exposed to cement, resulting in islands of decalcified zones or white spots. The reason for this variable susceptibility is unknown. Preliminary observations indicate that varnish is a protective to the surface of the enamel, hence its use should be advocated before placing orthodontic bands.

Grateful acknowledgement is made to Drs. D. Ziskin and E. Zegarelli of the Division of Oral Diagnosis of Columbia University, School of Dentistry for their invaluable assistance in examination and selection of specimens and performance of operative procedures.

REFERENCES TO LITERATURE

(1) Lefkowitz, W. and Bodecker, C. F., *J. D. Res.* **17**: 453, 1938.
(2) Mummery, S. P., *Proc. Royal Soc. Med.* **3**: 71, 1909.
(3) Bunting, R. W. and Palmerlee, F., *J. A. D. A.* **12**: 381, 1925.
(4) Enright, J. J., Friesell, H. E. and Trescher, M. O., *J. D. Res.* **12**: 759, 1932.

(5) Crowell, W. S., *J. A. D. A.* **14:** 1030, 1927.
(6) Paffenbarger, G. C., Sweeney, W. T., and Isaacs, A., *J. A. D. A.* **20:** 1965, 1933.
(7) Paffenbarger, G. C., Schoonover, J. C. and Souder, W., *J. A. D. A.* **25:** 32, 1938.
(8) Brill, J., *J. Den. Belge* **27:** 722, 1936.
(9) Titkemeyer, W., *Deutsch. Zhartze. Wochenschr.* **35:** 539, 1936.
(10) Noyes, H. J., *J. A. D. A.* **24:** 1243, 1937.
(11) Murless, F. T., Jr., *Internat. J. Orth.* **22:** 1248, 1936.
(12) Applebaum, E., Hollander, F. and Bodecker, C. F., *D. Cosmos.* **75:** 1097, 1933.
(13) Bodecker, C. F., *J. D. Res.* **10:** 481, 1930.
(14) Beust, T., *J. D. Res.* **11:** 619, 1931.
(15) Bodecker, C. F., *D. Cosmos* **71:** 586, 1929.
(16) Beust, T., *J. A. D. A.* **17:** 1536, 1930.
(17) Karshan, M. and Rosebury, T., *J. D. Res.* **14:** 220, 1934.
(18) Orban, B., *Dental Histology & Embryology* (Chicago, Rogers Printing Co.: 1928).

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THE KERATINIZATION OF THE HUMAN ORAL MUCOSA

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Earlier observations (1, 2) indicated the necessity for cytologic studies of the cellular elements in the saliva. The following report deals with the process of keratinization of the epithelial cells and with the degree of keratinization on different areas of the mucous membrane of the human mouth.

Smears were made from different areas of the mouth and stained without fixation. This method permitted the observation of each cell separately. This is the first time this method has been used for cytologic investigations of oral mucous membrane, insofar as the author has been able to determine.

REVIEW OF THE LITERATURE

The Process of Keratinization. Practically all studies concerning the mechanism of keratinization were made on the skin. The sole of the foot, where the keratinization is most distinct was frequently used. Cowdry (3) is of the opinion that the distance of the epithelial cells from the underlying dermis with its rich blood supply may have some relation to the process of keratinization due to a lack of certain essentials or an inability to eliminate waste products.

Many investigators believe that keratohyalin of the granular layer and eleidin of the stratum lucidum play an important role in the formation of keratin. MacLeod (4) considers keratohyalin as a separation product of cellular protoplasm that appears as vitality of the cell diminishes. Eleidin, a further product of the same substance, is probably the fatty or waxy substance which is present in the horny cells. Ludford (5) stated that the term eleidin has been applied to a substance said to resolve from the dissociation of keratohyaline. On the other hand Schaffer (6) and Patzelt (7) showed

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facts from which they concluded that there is no relationship between the formation of keratin and the existence of keratohyalin and eleidin.

The name of keratohyalin was introduced by Waldeyer (8). This substance is not only stained by basic dyes (methylene blue, fuchsin, safranin, gentian-violet) but by the acid dyes as well, (hematein, picrocarmin, waterblue, eosin, kongo red, acid fuchsin).

Various opinions have been expressed as to the origin of the keratohyalin granules. Mertschnigg (9), Posner (10), Selhorst (11), Laffront (12), Kreibich (13), Herxheimer and Nathan (14) and Ludford (5) described the keratohyalin originating from the nucleus. Ernst (15) and D'Urso (16) are of the opinion that keratohyalin is derived from the chromatin of the nucleus. Kromayer (17), Kollmann and Papin (18), and Patzelt (7) stated that keratohyalin is formed by the disintegration of the intraprotoplasmatic plexus of fibrils.

Rabl (19), Rosenstadt (20), and Martinotti (21) are of the opinion that the nucleus and cytoplasm both take part in the production of keratohyalin. While the keratohyalin granules characterize the stratum granulosum, the eleidin is included in the cells of the stratum lucidum. The cells of this layer are flatter than those of the underlying layer and some contain an empty cavity instead of a nucleus. Ehrmann and Fick (22) denied the existence of nuclei in this layer. According to Martinotti (21) the presence of the nucleus depends on the degree to which it participates in the formation of eleidin. When the nucleus begins to die, its reactions to certain stains change. No nuclei were observed in the stratum lucidum.

According to Hoepke (23) disintegration of the nucleus starts in the stratum granulosum and is completed in the stratum lucidum. Keratohyalin is found in granules of different sizes surrounding the nucleus. The cytoplasmatic content is basophil, the membrane amphophil. According to Unna and Schumacher (24) eleidin is replaced by keratin membrane in the higher layer of the stratum corneum. Unna (25) and Koelliker (26) described the cells of the stratum corneum as flat, plane scales with a membrane consisting of keratin. They found nuclear rest in some cells. Weidenreich (27) could not demonstrate nuclei in the cornified layer. He consistently observed cavities in the cells where the nuclei were formerly located. Merck (28) and Hoepke (23) believed that the nuclei still

existed but were not readily reactive to stain in such cases. Merck (28) was the first investigator to suggest that the cornified cells were not inert structures. Brock (29) confirmed this conception by clinical observations. Biedermann (30) concluded that the cornified cells must be lifeless since they are devoid of nuclei. Adler (31) and Hoepke (23) were of the opinion that cornified cells possess some manifestations of life, while Waldeyer (8) and Weidenreich (27) stated that the process of keratinization was a degenerative one, due to the drying of the skin. Hoepke (23) and Patzelt (7) stated that keratinization was a function of the cell.

The Keratinization of the Mucous Membrane of the Mouth. Severin (32) studied the mucous membranes of humans, oxen, swine, rabbit, rat, dog, cat and mole. He states that certain parts of the tongue are cornified. In such areas keratohyalin granules are found. These findings were confirmed by Posner (10). Both authors claim that the stratum corneum of the mucous membrane of the human mouth is not devoid of nuclei. Cells in this layer have pale thin nuclei. Ebner (33) writes that in the mouth keratinization is found on epithelium of the lip and one part of the tongue. All cells contain nuclei and the arrangement of the cells is very similar to that of the epidermis. Schumacher (34), Siegmund and Weber (35) are of the same opinion. Siegmund and Weber stated that under normal conditions a thin, shallow parakeratotic layer is formed. They found keratinization in cases of leucoplakia and black tongue.

Praeger (36) investigated the gum only and called the superficial layer "attrition layer." Bottler (37) is of the opinion that keratosis of the mouth occurs under pathologic conditions only and parakeratosis is normal, while keratinization of the skin is physiologic and parakeratosis is abnormal. He thinks this difference is due to the wetness of the mouth. Maximov (38) stated that the mucous membrane in the human mouth, under physiologic conditions, does not undergo cornification. He claims that the nucleus of the cells of the superficial layers shrinks and degenerates but does not disappear.

Gottlieb (39) claims that the gingiva is cornified under ideal conditions. Orban (40) demonstrated a keratinized layer on the gum containing epithelial cells devoid of nuclei. The characteristics of this layer varied. He found cells containing thin, pyknotic nuclei

in the stratum corneum, adjacent to areas in which the cells were free from nuclei. Empty vacuoles were observed in some of these cells. Keratohyalin granules were always seen in the stratum granulosum where normal keratinization took place. A stratum lucidum was never observed.

METHODS

Smears were taken by scraping different areas of the oral mucosa of 5 male and 5 female patients. The patients ranged from 25 to 40 years of age and presented healthy mouths.

The areas investigated were: the buccal folds in the region of the upper and lower first premolars, the alveolar gingivae, the hard palate, the tongue, and the cheek. In addition to these parts of the oral cavity a few smears from the soft palate and the floor of the mouth were studied. The entire number of smears studied was 737.

The end of an agate spatula which was 7 millimeters in width and 1 millimeter thick was used to scrape gently about 1½ square cm. of the surface of the mucosa. The material was carefully smeared on two separate slides. If it was not handled gently, the cells were crushed.

The smears were dried for two to three minutes, then they were stained by Wright's modification of Leishmann's method, (41), and by the Ernst-Gram method. Other types of stains were used, but the results were not as satisfactory as the routine methods. Cells in smears fixed with 5 per cent formalin, formalin vapor at room temperature, absolute alcohol, Zenker formalin, acetic acid Zenker, or Bouin's fluid revealed more shrinkage than cells in unfixed smears. Smears stained with Delafield's, Heidenhain's or Erlich's hematoxylin; Masson's trichrome stain, Papanicolaou's method or Mallory's stain showed no advantages over the Wright or Gram stains.

The following procedure for Gram's staining was found to be the best:

Solution

I. Saturated alcoholic solution of gentian-violet (Gruebler)	10 cc.
5 per cent aqueous solution of Phenol	90 cc.
II. Lugol's Solution	
Iodine	1 gram
Potassium Iodide	2 grams
Distilled water	300 cc.

Method

1. The preparation was covered with solution I for thirty seconds.
2. Solution II for thirty seconds.
3. Wash with 95 per cent alcohol until the color ceases to come out of the preparation.
4. Wash in water for two or three seconds.
5. Safranin for two minutes.
6. Wash in water for two or three seconds, dry, add Canada balsam, and cover glass.

FINDINGS

Region of the cheek. The striking impression gained by examination of the smear from this area is the relatively great number of

leucocytes in comparison to the number of epithelial cells. Sometimes we found more leucocytes than epithelial cells. There were a great number of well preserved leucocytes. We found many cells in which the nucleus and the granulated cytoplasm were distinctly stained. The majority however showed signs of degeneration of different degrees.

The smear stained by Wright's method showed a rather uniform picture under low magnification. The epithelial cells were separated from one another or grouped in small and large plaques. Almost all epithelial cells contained stained nuclei and were about the same size, one diameter a little longer than the other (about 100 x 80 microns). Their shape varied from four cornered to heptagonal. Most of them had six angles.

The nuclei had a sharp outline, were stained blue or purple, showed a granular feature and contained two or three vacuoles. Their shape varied from oval to round, though they showed a deviation in shape and in reaction to the Wright stain. We were not able to differentiate distinct types of nuclei. There was only the general impression: the smaller the nucleus the darker it was stained. Sometimes cells with two nuclei were found. Such cells were bigger than cells with a single nucleus. The nuclei were frequently the same size, but sometimes one was larger than the other.

The cytoplasm was stained pale pink but not evenly, some areas taking a little more dye than others. No rule could be found by which the various cells could be differentiated by the relation to Wright's stain.

Most of the smears from the cheeks stained by the Ernst-Gram method showed a similar picture to that described above. This stain differed from Wright's in that the nucleus and cytoplasm were stained red, the nucleus darker than the cytoplasm. The description of the epithelial cells stained by the Ernst-Gram method was generally the same as those stained by Wright's method. The epithelial cells described above will be classified as Type A in this discussion for convenience. Type A cells are characterized by a red nucleus and cytoplasm when stained by the Ernst-Gram method. The nuclei showed some variation in size and shape (*fig. 1*).

In a few smears from the cheek, epithelial cells were found in which

the nuclei were partly or wholly stained by gentian violet. The nuclei contained irregular blue granules that in some cases were separate and distinct, leaving the greater portion of the nuclei stained red. In other nuclei the granules had fused together forming larger irregular blue staining masses, while others had nuclei in which the red staining characteristic was eliminated entirely leaving the nucleus blue. Cells with these staining characteristics were called Type B (*fig. 1*).

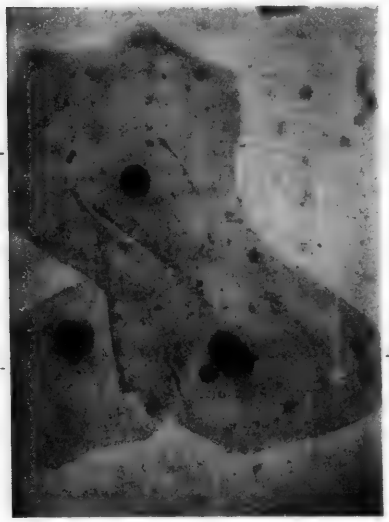


FIG. 1

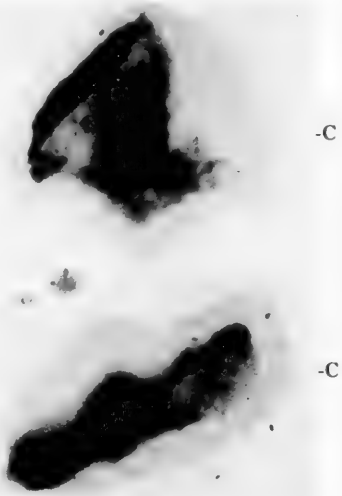


FIG. 2

FIG. 1. Epithelial cells from the region of the cheek. Magnification approx. × 650. Gram's method. The lower left cell: nucleus and cytoplasm are stained red—Type A. The two other cells: nucleus stained by gentian-violet, cytoplasm red—Type B.

FIG. 2. Two epithelial cells from the region of the cheek. Magnification approx. × 650. Gram's method. The nucleus and part of the cytoplasm are stained by gentian-violet—Type C.

In addition to the types described above, epithelial cells were observed in which both the nucleus and parts of the cytoplasm were stained by gentian violet. The contour of the nucleus was distinguishable. The blue stained granules varied in size from fine powderlike inclusions to fused droplets. They surrounded the nucleus, but sometimes they were separated from it by varying amounts of red stained cytoplasm. These cells were called Type C (*fig. 2*).

Resumé of cheek region. The smears from the cheek were characterized by numerous leucocytes and epithelial cells all containing nuclei. While the smears stained with Wright's do not permit classification of the epithelial cells, the Ernst-Gram stains show three different types in some specimens. The first, Type A, contained nuclei and were stained red throughout. The second, Type B, contained nuclei that were partly or wholly blue with red cytoplasm. The third, Type C, contained nuclei that were entirely blue and cytoplasm that was partly blue.

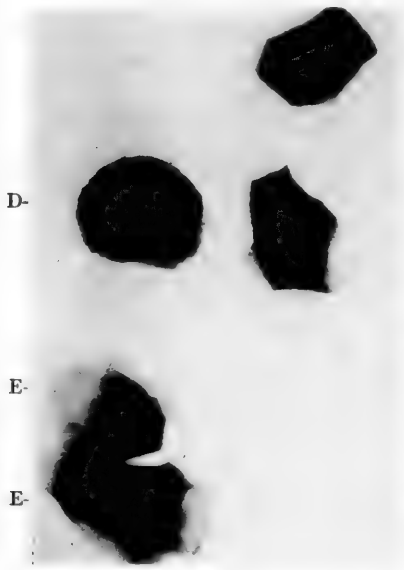
Upper buccal fold. The smears from this area contained fewer leucocytes than those taken from the cheek in the great majority of cases. The epithelial cells were very similar to those in the smears from the cheek. The predominant cell was Type A. In some smears however, Type B and Type C were equal in number or predominant. No essential differences were found between smears taken simultaneously of corresponding areas from the same individual.

Tongue. Smears taken from the posterior two-thirds of the tongue were characterized with Wright's stain by comparatively small numbers of leucocytes and many epithelial cells. About the same number of cells were found with and without nuclei.

The cells with nuclei stained equally well with Wright's or Gram-Ernst and resembled those described in the smears from the cheek. In the specimens stained with Gram-Ernst we found cells of Type A, B, and C. Cells without nuclei were a great deal smaller than cells containing nuclei. They were stained almost homogenously blue with Wright's. Sometimes the cytoplasm appeared much darker blue than the cytoplasm in cells containing nuclei.

Smears from the tongue stained by the Ernst-Gram method made possible the classification of two additional types of epithelial cells without nuclei. Some of the cells were stained a deep dark blue by gentian violet. Almost no structural detail could be distinguished. These cells were called Type D (*fig. 3*). In other cells with no nucleus we differentiated two portions with variable staining characteristics. The larger central portion was blue, the smaller peripheral portion pink. The stain in both areas was not homogenous. In the blue stained portion there were irregular pink spots, while in the pink, light blue strands were seen. The picture in the blue stained area

resembled an eroded stone. Comparison of a great number of these cells indicated that they might represent different stages of cornification. In the first stages the pink stained portion occupied only a small part of the cellular area. In the more advanced stages the greatest part of the cell was stained pink, while only small areas of



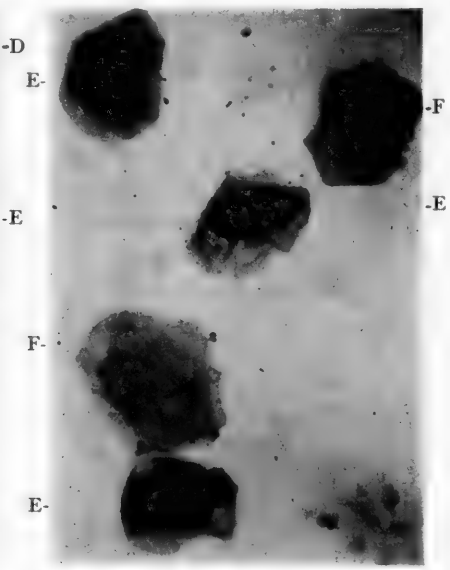


FIG. 3 FIG. 4

FIG. 3. Five epithelial cells from the tongue. Magnification approx. × 465. Gram's method. The right upper and the left center cell are entirely stained by gentian-violet, nuclei are non-distinguishable—Type D. Only parts of the other cells are stained by gentian-violet. Nuclei are non-distinguishable—Type E.

FIG. 4. Five epithelial cells from the gingivae. Magnification approx. × 445. Gram's method. The entire body is stained by safranin, nuclei are non-distinguishable—Type F. Three different stages of Type E.

blue stained granules or strands remained. This type of epithelial cell characterized by lack of nucleus and stained partly by safranin, partly by gentian violet was called Type E (*figs. 3 and 4*).

Gingivae. The smears from this area were characterized by the almost complete absence of leucocytes and epithelial cells of Type A. There were very few epithelial cells of Type B. The predominant

epithelial cells were of Type C, D, and E. In some specimens stained by the Ernst-Gram method a sixth cellular type was seen. These cells were without nuclei, the entire cell was stained by safranin and no part of the cell was stained by gentian violet. This type was called Type F (*fig. 4*).

Hard palate. The smears from this area were characterized by complete absence of leucocytes and the rare appearance of epithelial cells showing nuclear structure Type A, B, and C. The predominant type was D; Types E and F were present in smaller numbers.

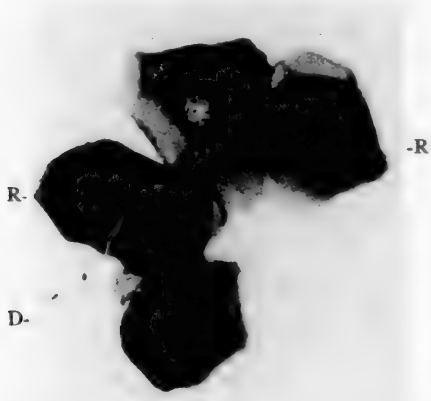


FIG. 5. Area of the hard palate. Magnification approx. × 535. Stained by Gram's method. The three upper epithelial cells, ring-cell type, are labelled R. The lower cell—Type D.

In addition to the types described, large numbers of epithelial cells were observed that had certain characteristics when they were stained by Wright's method. Three zones were distinguishable. In the center, where one would ordinarily expect to find the nucleus, an area was seen that was stained much lighter than the other parts of the cell. This area, in its size and shape, corresponded to the form of a nucleus. The lack of dye indicated that there was less chromatin present. Due to this condition the cell had the appearance of a ring. Toward the outside of the cell the light stained area was surrounded by a broad deeper staining stripe, in which very fine vacuoles were

seen. When the fine adjustment was moved these vacuoles appeared as dark fine granules.

This second area did not reach the periphery of the cell at all points. The remaining peripheral portion stained lighter and was more homogenous than the broad, deep staining circular stripe. Specimens from the same source stained by the Ernst-Gram method showed cells with similar staining characteristics. A central pale field was separated from the peripheral dark stained stripe by a thin light red layer (*fig. 5*).

DISCUSSION

It is a well-known fact that the oral cavity is lined with squamous epithelium. There is a question regarding the ability of this epithelium to produce a keratinized layer under normal conditions. By gently scraping the surface of the oral mucous membrane according to the method described above we were able to study those cells that are easily obtained from the superficial layers of the oral mucosa. Although this method has been used for the study of the mucosa in other parts of the body, the author was unable to find references in the literature to its use in the mouth (Stockard and Papanicolaou, Herrenberger).

The smear method has definite advantages over the usual histological method in that the same area may be investigated repeatedly without injuring the tissue. The cells may be studied individually and artifacts are reduced to a minimum. The disadvantage, that the cells are not in anatomical relationship, can be overcome by comparison of the smears with histologic sections of corresponding areas.

Ernst found that substances in the process of keratinization stain readily with gentian violet but completely keratinized substances do not stain with this dye. Specimens of cornified epithelium stained by the Ernst-Gram method show three layers. The first, adjacent to the connective tissue of the submucosa is stained red and the cells have nuclei. The second is stained blue by gentian violet. The third is stained red, as is the first layer, but the nuclei are either absent or they are replaced in some instances by light staining areas. There are no sharp lines of demarcation separating the three layers, but rather a gradual blending of each of the zones into one another.

The middle layer according to its staining reaction is composed of those cells which are in the process of keratinization. The third or superficial layer is composed of cells completely cornified. The method applied in this report permitted the study of the intermediate changes in detail and of the differentiation of distinct cell types.

We were able to differentiate by the use of the Ernst-Gram method six stages of cornification of epithelial cells.

Type A—Nucleus and cytoplasm were not stained by gentian violet.

Type B—The nucleus was partly or entirely stained by gentian violet.

Type C—Nucleus and parts of the cytoplasm were stained by gentian violet.

Type D—Nucleus was not distinguishable. The entire cell was stained by gentian violet.

Type E—Nucleus was not distinguishable. The cell was stained by gentian violet with the exception of a peripheral portion.

Type F—Nucleus could not be recognized. No part of the cell was stained gentian violet.

The smears from the region of the cheek showed cells with well-preserved nuclei. Neither the cytoplasm nor the nucleus was stained by Gram's method. In only a few cases cells were observed in which the nucleus and sometimes parts of the cytoplasm showed affinity for gentian violet. The nucleus was entirely or partly stained by gentian violet when the cytoplasm was stained. However, there were cells where only the nucleus showed affinity to this dye. This observation might indicate that in the course of changes leading to keratinization, the nucleus is the first part of the cell that exhibits changes in staining characteristics. If the process progresses farther, then the cytoplasm is also affected. No specimen from the cheek indicated a complete keratinization in this region. The majority of the cells studied from the cheek showed no changes that could be interpreted as a stage preceding the stage of keratinization. Nevertheless, in some instances cells were found where the staining reaction indicated the first signs of keratinization. We assumed that the epithelial cells in the area of the cheek were sloughed off before the cells were able to produce keratin.

On the tongue the cells were probably sloughed at a later stage of cornification. In addition to the types seen in smears from the cheek, cells were observed in which parts of the cytoplasm and in some instances the entire cell was stained by gentian violet. If the whole cell body was stained blue, the structure of the nucleus could not be distinguished. A further stage toward complete keratinization was seen in that cell type in which the peripheral portion did not take the Gram stain and the nucleus was not apparent.

If we accept the general conception of keratinization, characterized by the lack of nuclei, and by the complete affinity to acidophil dyes, we have to state that the tongue is not completely keratinized but that it is keratinized to a higher degree than the mucous membrane of the cheek.

Cells were regularly found in the region of the gum in which all the requirements of complete keratinization were demonstrated. This observation was in accord with those of Orban who pointed out that the gingivae showed true cornification under normal conditions. Cells described as Types A and B from smears of the tongue and cheek were missing in smears from the gum.

The highest degree of keratinization of the oral epithelium studied was seen in smears from the mucous membrane of the hard palate. Cells in which a nucleus could be recognized were very rare. The majority of cells belonged to Type E (cells where no nucleus could be noticed and where the entire cell body was stained by gentian violet). The other cells in smears from this region contained no nuclei and were partly or entirely non-reactive to Gram's stain (Types E and F).

These studies demonstrated the variation in the staining characteristics of epithelial cells taken from the surface of different areas of the mouth. From these observations and those of Ernst we may conclude that the histological structure of the mucous membrane varies in different areas. The mucosa of the cheek showed no keratinization. The mucous membrane of the hard palate was completely cornified and the mucous membrane of the tongue lies between the two extremes.

On the other hand, the staining characteristics of epithelial cells in smears taken repeatedly from corresponding areas of the mouth varied to a very small degree.

In addition to the six types mentioned above another type of epithelial cells was observed. These cells were characterized by the fact that a lighter stained area corresponding to the size of the nucleus appeared in that portion of the cell where the nucleus is usually seen, thereby giving the cell a ring-like appearance. Most of these cells were stained by Gram's method, but sometimes they were not reactive to gentian violet, and were stained by the counterstain. In both cases the area of the nucleus took less dye than the rest of the cell body. These cells corresponded to those which were described by several authors (27, 28, 23) and were found in the cornified layer of the epithelium. The studies of the author did not contribute essential facts to the genesis of this type of cell. For this reason it was not possible to ascertain the position of these ring cells in the life cycle of the squamous epithelium.

From a teleological point of view cornification is considered a protective phenomenon. From a functional aspect keratinization may be explained as the reaction of the squamous epithelium to different stimuli and irritations. There are studies, however, that indicate the existence of another defense mechanism which may replace keratinization.

A large number of leucocytes were observed in the area of the cheek mucosa which is normally not keratinized. On the keratinized mucous membrane of the hard palate no leucocytes were seen. A relation between keratinization and the number of leucocytes has been observed by other investigators. In cases of gingival infiltration Wolpe (42) failed to demonstrate the presence of a keratinized layer of the epithelium, while under normal conditions a keratinized epithelium and only a few leucocytes were present. He explained both cornification and infiltration by leucocytes as a defense reaction. Ziskin (43) demonstrated an increase of keratinization of the alveolar gingivae and decrease of inflammatory reaction in the sub-epithelial layer due to injection of estrogenic preparations. A similar relationship may be seen in his studies of the gingivae during pregnancy (44). Lewis (45), influenced by experiments of Allen, demonstrated an increase in the thickness of the vaginal epithelium and simultaneously a disappearance of leucocytes associated with the therapeutic injection of theelin. Herrenberger (46) confirmed these observations

and claimed that the number of leucocytes were in inverse proportion to the development of vaginal epithelium. He found many leucocytes as long as the formation of the vaginal epithelium was not completed. However, after puberty vaginal smears were free from leucocytes. Papanicolaou (47) and Murray (48) described an increase of the number of leucocytes in cases of atrophic vaginal mucosa.

These observations and those of the author lead to the assumption that keratinization and leucocytic activity are biological defense mechanisms of the body.

It seems that where the surface of the mucous membrane is not protected by a keratinized layer, the migrating leucocytes take over the function of protection.

SUMMARY

Smears taken from the surface of the mucous membrane of the mouth were used to study the cytologic detail of the superficial epithelial cells. The staining method of Ernst-Gram revealed a series of six stages in the process of keratinization. The degree of keratinization was lowest in the smears from the cheek; it increased progressively in the smears from the tongue, gingivae and palate. The number of leucocytes in the smears decreased gradually with an increase in the degree of keratinization of the epithelial cells.

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REFERENCES TO LITERATURE

(1) Weinmann, J., *J. D. Res.*, **15**: 360, 1935–1936.
(2) Orban, B., and Weinmann, J., *J. A. D. A.*, **26**: 2008, 1939.
(3) Cowdry, E. V., *Special Cytology* (New York, Hoeber: 1932), p. 18.
(4) MacLeod, J. M. H., *Mon. f. prakt. Dermatol.*, **28**: 37, 1899.
(5) Ludford, R. J., *Quart. J. Micr. Sc.*, **69**: 27, 1925.
(6) Schaffer, I., *Lehrbuch der Histologie und Histogenese*, (Leipzig, 1937).
(7) Patzelt, V., *Jahrb. f. Morphol. u. Mikrosk. Anat.*, Abt. 2, **5**: 371, 1926.
(8) Waldeyer, W., *Beitr. z. Anat. u. Embryol.*, (Henle Festgabe, 1882), p. 141.
(9) Mertschnigg, *Virchow's Archiv. f. pathol. Anat. u. Physiol.*, 116: 484, 1889.
(10) Posner, C., *Virchow's Archiv.*, **118**: 319, 1889.
(11) Selhorst, S. B., *Ueber das Keratohyalin und den Fettgehalt der Haut* (Berlin, O. Francke: 1890).
(12) Laffront, A., Quoted from Hoepke (23).
(13) Kreibich, C., *Arch. f. Dermatol. u. Syphilis*, **121**: 313, 1915.
(14) Herxheimer, K., and Nathan, M., *Arch. f. Dermatol. u. Syphilis*, **123**: 76, 1916.

(15) Ernst, P., *Virchow's Archiv f. pathol. Anat. u. Physiol.*, 130: 279, 1892.
(16) D'Urso, Quoted from Hoepke (23).
(17) Kromayer, F., *Dermatol. Ztschr.*, 4: 335, 1897.
(18) Kollmann, M., and Papin, L., *Arch. d'Anat. Micro.*, 16: 193, 1914.
(19) Rabl, H., *Ztschr. f. d. ges. Anat.*, Abt. 3: *Ergebnisse d. Anat. u. Entwicklungsgesch*, 7: 339, 1897.
(20) Rosenstadt, B., *Arch. f. mikrosk. Anat.*, 91: 182, 1918.
(21) Martinotti, L., *Arch. f. Zellforschung*, 13: 446, 1915.
(22) Ehrmann, S., and Fick, J., *Einfuehrung in das mikroshop. Studium der normalen und kranken Haut* (Wien, A. Hoelder: 1905).
(23) Hoepke, H., *Handbuch der Mikropischen Anatomie des Menschen. Die Haut* (Berlin, Springer: 1927) 3: 1.
(24) Rabl, H., *Histologie der normalen Haut des Menschen.* in Mracek, *Handbuch d. Hautkrankh.* (Wien, A. Hoelder: 1901).
(25) Unna, P. G., *Entwicklungeschichte und Anatomie der Haut* in Ziemmissens' *Handb. d. spec. Pathol. u. Therapie.* (Leipzig, 1883).
(26) Koelliker, Albert, *Handbuch der Gewebelehre* (Wien, W. Engelmann: 1889).
(27) Weidenreich, *Arch. f. Mikrosk. Anat.*, 57: 583, 1901.
(28) Merk, L., *Arch. f. Mikrosk. Anat.*, 56: 525, 1900.
(29) Brock, *Arch. f. Dermatol. u. Syphilis*, 45: 369, 1898.
(30) Biedermann, W., *Physiologie der Stuetzsubstanzen* in *Handb. d. vergl. Physiol.* von Winterstein (Jena: 1914) 3: 319.
(31) Adler, N., Quoted from Hoepke (23).
(32) Severin, *Arch. Mikrosk. Anat.*, 26: 81, 1886.
(33) Ebner, V., *Koelliker's Handbuch der Gewebelehre des Menschen.* 6 Auflage (Wien, W. Engelmann: 1902) 3: 9.
(34) Schumacher, S., *Z. Anat.*, 73: 247, 1924.
(35) Siegmund, H., and Weber, R., *Pathologische Histologie der Mundhoehle* (Leipzig, Hirzel: 1926).
(36) Praeger, W., *Vjschr. f. Zahnheilkunde*, 2: 225, 1927.
(37) Bottler, R., *Deutsch. Zahnaerztl. Wochenschr.*, 12: 615, 1930.
(38) Maximov, A. A., and Bloom, W., *A Textbook of Histology* (Philadelphia, Saunders: 1938) p. 345.
(39) Gottlieb, B., *Z. f. Stomat.*, 3: 129, 1921; *D. Mon. f. Zhk.*, 5: 142, 1921; *Fortschr. d Zhk.*, 3: 366, 1927.
(40) Orban, B., *Ztschr. f. Anat. u. Entwicklungs.*, 94: 25, 1931.
(41) Mallory, F. B., and Wright, J. H., *Pathological Technique* (Philadelphia, Saunders: 1918).
(42) Wolpe, P., *Deutsch. Mon. f. Zhk.*, 54: 1, 1929.
(43) Ziskin, D. E., *J. A. D. A.*, 25: 422, 1938.
(44) Ziskin, D. E., Blackberg, S. N., and Stout, A. P., *Surgery, Gynecology and Obstetrics*, 57: 719, 1933.
(45) Lewis, R. M., *Amer. J. Obstetr.*, 26: 593, 1933; 29: 806, 1935.
(46) Herrenberger, K., *Arch. Gyn.*, 167: 506, 1938.
(47) Papanicolaou, G. N., and Shorr, *Proc. Soc. Exper. Biol. A. Med.*, 32: 585, 1935.
(48) Murray, E. G., *Arch. Gyn.*, 165: 653, 1938.

A METHOD OF PLASMA PHOSPHATASE DETERMINATION FOR DIFFERENTIATION OF ALVEOLAR CREST BONE TYPES IN PERIODONTAL DISEASE[1]

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INTRODUCTION

The importance of loss of teeth from periodontal involvement is gaining ever greater recognition due both to its very common occurrence and to the broadening horizons of knowledge in the field of periodontia. Information concerning the fundamental biochemical changes underlying or accompanying the alveolar crest bone deviations observed clinically (as indicated by marked mobility of a given tooth) or radiographically, unfortunately, remains meagre. The complexities inherent in such studies make for difficult solution.

PURPOSE OF THE INVESTIGATION

This study in plasma phosphatase variation in relation to alveolar bone loss was undertaken: 1. To trace such changes in phosphatase value as occur both locally in the region of various types of alveolar bone decalcification and in the blood plasma from the finger, and 2. To evaluate the validity of such phosphatase values as an index of rapidity of alveolar bone loss in comparison to radiographic findings.

RADIOGRAPHIC CLASSIFICATION OF ALVEOLAR CREST BONE TYPES

The 'normal' alveolar crest should reach an expected level interproximally in relation to the teeth that is determined by the age of the patient (1); the unchanging distribution of trabeculations and medullary spaces of the alveolar bone as seen radiographically should be

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covered at the apex by an even and unbroken thickness of lamina dura. Miller (2) has classified several types of alveolar bone. Alveolar crests that show a radiographic picture similar to the normal, though terminating at a level lower than the expected normal, indicate a condition of 'arrested' bone destruction or healing following a disease process that had been manifested in the alveolar bone.

With disease of the supporting structures of the teeth, the early radiographic change of thickening of the peridental membrane may be followed by alveolar crest destruction which can be differentiated into 'slow' and 'rapid' types. If the dissolution of the alveolar crest tends to terminate abruptly, with sharply demarcated normal underlying or supporting alveolar bone, the region may be regarded as one in which 'slow' destruction is occurring. The radiographic picture of the 'rapid' type of alveolar crest destruction shows a more deepened decalcification of the crest and, rather than a sharp line of demarcation with the underlying bone, there is presented a gradual blending of the area of destruction into the normally calcified supporting alveolar bone. Greater explanatory detail for differentiation, and illustrations of the various alveolar types, can be found elsewhere (3).

THE ENZYME PHOSPHATASE

The presence of phosphatase in the blood plasma was first demonstrated in 1924 by Maitland, Hausman and Robison (4). The enzyme action of phosphatase is held to be the cause of calcification of bone through inducing supersaturation of calcium phosphate in the fluid around the cartilage cells (5). It acts by hydrolyzing some phosphoric ester, such as hexosemonophosphate, glycerophosphate, etc., leading, according to Kay, to "a local production in the calcifying zone of an inorganic phosphate ion concentration considerably higher than that in the blood"(6).

Deposition of calcium phosphate occurs when the concentration of $Ca^{++} \times PO_4^{=}$ exceeds the solubility product of the salt, i.e., $CaHPO_4$ or $Ca_3(PO_4)_2$. Phosphatase is believed to be the product of the osteoblasts, the proliferating cartilage cells, and the cells of the inner layer of the periosteum (7). Normally, the enzyme is found in highest concentration in ossifying cartilage, in lower concentration in formed bone, and is absent from resting epiphyseal cartilage and non-

ossifying cartilage. The amount is greater in rapidly growing bone and tooth than in any other structure.

The optimum rate of phosphatase activity is usually reached in the neighborhood of pH 9 and is readily inactivated by acids at pH 5 or below. Bone is held to be either the main or sole source of plasma phosphatase. A marked increase in value occurs in obstructive jaundice since the enzyme is excreted by the liver, but the phosphatase reaction of the plasma is not affected to any extent by the removal of such organs as the kidney, pancreas, spleen, etc. Increased plasma phosphatase in obstructive jaundice has been demonstrated by Roberts (8) and with changes in catarrhal jaundice, supports the hypothesis of the hepatogenic source of serum phosphatase (9).

Plasma phosphatase is markedly increased in certain generalized bone disease such as osteitis deformans, generalized osteitis fibrosa, osteomalacia, rickets, etc. Various views have been advanced to explain this pathological increase. Some of these are: 1.—Increase in phosphatase is an index of greater activity of the bone cells with the increase in cellular activity either osteoblastic or osteoclastic in nature (10). 2.—Phosphatase value is highest when there is excessive formation of abnormal osseous tissue. Bone destruction will not give marked rise in plasma phosphatase unless new bone or osteoid tissue is being formed. Variations in plasma phosphatase may be due to variations in the cellular activities of the bone cells which may be controlled by certain factors that function in the normal development of bone and probably undergo alteration when disease processes occur (11). 3.—"It may be that it (phosphatase) is produced in excessive amounts in the bone as an attempt on the part of the organism at compensation for the lesion, or possibly, owing to the weakness of the bone, there is a greater amount of bending and crushing which mechanically squeeze out some of the cell contents" (6).

Labbé (12) presented an interesting study on variation in phosphatase concentration in the alveolar bone of fresh cadavers. Where evidence of alveolar bone destruction was present, 2 to 5 times the phosphatase concentration was found in the alveolar bone as compared to specimens of bone from the tibia similarly tested. Such increase of phosphatase concentration can be explained either by assuming a defensive action of the phosphatase to induce calcification and coun-

teract the destructive process or as being the result of phosphatase loss from the disintegrating bone cells.

METHOD EMPLOYED IN PLASMA PHOSPHATASE DETERMINATION

All methods of phosphatase estimation are based on its action in vitro of hydrolyzing phosphoric esters to free an increased quantity of inorganic phosphate. The phosphate value of a given sample taken before and after the action of the enzyme has been permitted to take place on a suitable substrate, for a fixed period of time and at a constant temperature, yields the index of phosphatase activity.

The nature of the marginal gingival circulation is such as to make it almost impossible to withdraw sufficient blood for the usual methods of estimation of phosphatase activity [Bodansky (13) and Kay (14)]. It was necessary, therefore, to employ a method utilizing smaller quantities of blood plasma in the determination. The literature was found to give information on the required micromethod as developed by Lundsteen and Vermehren (15).

The procedure, as modified for the present purpose, follows:

The gingival tissues over the alveolar area selected for investigation were dried carefully, and the interproximal papilla removed by a sharp scalpel to the height of the alveolar bone, inducing the least possible trauma. Fifty cubic mm. of the resultant blood in the area was drawn directly into a small measuring pipet and washed into 1,000 cubic mm. of a 0.9 per cent sodium chloride solution. The corpuscles were centrifuged out, a clear supernatant liquid sufficient for two blank experiments remaining.

Two hundred cubic mm. of the supernatant liquid were added to 200 cubic mm. of substrate (made up of 8 cc. of N ammonium hydroxide +12 cc. of N ammonium chloride + 1 gm. of disodium B glycerophosphate + 2 cc. of M magnesium chloride; to 100 cc. of water). The solution was permitted to incubate in a constant temperature water bath for 24 hours at 37°C. The reaction was then stopped by adding 300 cubic mm. of 10 per cent trichloracetic acid. A blank determination was carried out simultaneously, in which 200 cubic mm. of enzyme solution, 200 cubic mm. of substrate and 300 cubic mm. of trichloracetic acid were mixed without the intermediate incubation. The precipitated protein matter was centrifuged out from each, and a phosphorus determination carried out with the clear supernatant liquid. Four hundred cubic mm. were removed and 100 cubic mm. of sulphuric acid-molybdate solution (100 cc. of 7.5 per cent ammonium molybdate solution + 45 cc. of 10 N sulphuric acid + 105 cc. of water) and 100 cubic mm. of amidol solution (15 gm. of sodium sulphate + 15 gm. of amidol in 100 cc. of water, diluted five times with water before use) were added. The intensity of the blue color which developed in 15 minutes was determined by a Pulfrich Photometer with 10 mm. cells in the micro-attachment, using red filter No. 72. The difference in the amounts of phosphorus in the analysis and the blank experiment was then the amount of phosphorus which had been liberated by the phosphatase.

TABLE I

Phosphatase values in radiographically 'normal' alveolar crest bone

SPECIMEN NUMBER	AGE OF PATIENT	SOURCE OF SPECIMEN	ORIGINAL P UNITS	P UNITS AFTER INCUBATION	CALCULATED PHOSPHATASE	DIFFERENCE IN PHOSPHATASE VALUES
88	22	Gingiva	4.2	13.1	8.9	−1.6
89		Finger	4.2	14.8	10.6	
68	16	Gingiva	5.9	22.2	16.3	−1.3
69		Finger	5.6	23.3	17.7	
62	34	Gingiva	4.5	12.8	8.3	−1.3
63		Finger	4.5	14.1	9.6	
80	35	Gingiva	4.8	11.7	6.8	−0.6
81		Finger	4.3	11.6	7.4	
33	28	Gingiva	8.0	17.7	9.7	0.0
34		Finger	7.4	17.0	9.7	
78	29	Gingiva	3.8	17.1	13.4	+0.1
79		Finger	3.6	16.9	13.3	
163	42	Gingiva	4.1	24.6	20.4	+0.6
164		Finger	4.4	24.2	19.8	
123	22	Gingiva	4.9	15.4	9.5	+0.6
124		Finger	5.1	14.0	8.9	
31	42	Gingiva	7.1	15.1	8.0	+0.6
32		Finger	7.6	15.0	7.4	
117	28	Gingiva	3.9	12.9	9.0	+1.0
118		Finger	4.0	11.9	8.0	
39	38	Gingiva	12.9	32.3	19.4	+1.0
40		Finger	12.7	31.2	18.4	
165	55	Gingiva	5.6	23.8	18.2	+1.2
166		Finger	4.7	21.7	17.0	
72	22	Gingiva	3.5	14.0	10.5	+1.4
73		Finger	3.4	12.5	9.1	
115	32	Gingiva	14.5	23.4	8.8	+1.8
116		Finger	14.4	21.4	7.0	
152	40	Gingiva	4.6	14.2	9.6	+3.0
153		Finger	4.2	11.2	6.6	
35	30	Gingiva	6.5	31.3	24.8	+3.6
36		Finger	6.4	27.6	21.2	

Since the amount of phosphorus liberated is proportional to the amount of phosphatase, the unit chosen in this method is the amount of phosphatase which, in 24 hours at 37°C., will liberate 1 mg. of phosphorus from the given substrate. "If the amount of phosphorus liberated in the 400 cubic millimeters used in the phosphorus estimation is termed a, and the fraction of the whole blood constituted by plasma, pl, then the total amount of phosphorus liberated per hundred cubic centimeters of plasma after a reaction time of twenty-four hours is given by:

$$a\left(\frac{700}{400}\right)\left(\frac{1050}{200}\right)\left(\frac{100}{50\,pl}\right) = \frac{a}{pl}\,18375 \text{ mg. P (reference 15)"}$$

The plasma determination was taken by means of a finger blood sample as described by Van Allen (16). A similar procedure was carried out with a blood sample from a finger prick of the same patient. The difference, therefore, between the plasma phosphatase value of the blood in the region of the alveolar bone and of the blood plasma of the patient's finger would indicate the deviation from the normal due to the progressive de structive process.

PLASMA PHOSPHATASE FINDINGS FROM ALVEOLAR BONE TYPES

The phosphatase data collected was divided into 4 groups on the basis of the radiographically indicated alveolar crest types earlier described. Indication of positive or negative differences in plasma phosphatase values was in all cases made by arbitrarily designating the findings from the finger blood as the subtrahend and expressing the remainder as the difference. Arrangement of data was in relation to sequence of such numerical phosphatase value differences.

Table I presents the phosphatase values resulting from testing of the radiographically indicated 'normal' and 'arrested' alveolar bone types. In each instance, the increase in phosphorus concentration after incubation of the gingival blood sample and the finger blood sample permitted for calculation in equivalent phosphatase units. The difference between the calculated phosphatase value of the 2 blood samples gives indication of such plasma phosphatase variation as may have occurred in the region of the alveolar bone in contrast to the individual normal finger blood phosphatase value. Such differences here vary from -1.6 to $+3.6$ phosphatase units, with a mean difference of $+0.6$ units.

Table II indicates the plasma phosphatase findings from a series of interproximal areas in which the underlying alveolar crest bone showed the described radiographically indicated 'slow' type of alveolar bone dissolution. A uniform increase in the phosphatase values of

TABLE II

Phosphatase values in radiographically indicated 'slow' alveolar crest dissolution

SPECIMEN NUMBER	AGE OF PATIENT	SOURCE OF SPECIMEN	ORIGINAL P UNITS	P UNITS AFTER INCUBATION	CALCULATED PHOSPHATASE	DIFFERENCE IN PHOSPHATASE VALUES
92	32	Gingiva	5.6	19.2	13.6	+4.3
93		Finger	5.1	14.4	9.3	
56	47	Gingiva	7.4	16.7	9.2	+4.4
57		Finger	7.6	12.4	4.8	
58	30	Gingiva	7.8	21.2	13.4	+4.7
59		Finger	7.6	16.3	8.7	
102	23	Gingiva	5.0	15.4	10.4	+5.2
103		Finger	4.9	10.1	5.2	
100	37	Gingiva	7.4	17.3	9.9	+5.5
101		Finger	7.2	11.5	4.4	
143	22	Gingiva	5.0	14.4	9.4	+5.5
145		Finger	6.3	10.2	3.9	
29	60	Gingiva	6.7	21.9	15.2	+5.6
30		Finger	7.3	16.9	9.6	
54	28	Gingiva	6.1	18.8	11.5	+5.7
55		Finger	7.2	12.0	5.8	
104	29	Gingiva	6.2	18.0	11.8	+5.8
105		Finger	6.5	12.5	6.0	
70	36	Gingiva	4.7	23.9	19.2	+5.9
71		Finger	4.6	17.9	13.3	
94	25	Gingiva	5.1	23.7	18.6	+6.1
95		Finger	4.9	17.5	12.5	
131	41	Gingiva	6.5	19.7	13.2	+7.2
132		Finger	6.5	12.5	6.0	
126	41	Gingiva	5.2	20.4	15.2	+7.3
128		Finger	6.2	14.2	7.9	
136	21	Gingiva	5.7	23.1	17.4	+7.9
138		Finger	6.2	15.7	9.5	
25	52	Gingiva	8.8	25.7	16.9	+11.9
26		Finger	11.6	16.6	5.0	
66	41	Gingiva	5.4	28.2	22.8	+13.6
67		Fingers	4.3	13.5	9.2	

TABLE III

Phosphatase values in radiographically indicated 'rapid' alveolar crest dissolution

SPECIMEN NUMBER	AGE OF PATIENT	SOURCE OF SPECIMEN	ORIGINAL P UNITS	P UNITS AFTER INCUBATION	CALCULATED PHOSPHATASE	DIFFERENCE IN PHOSPHATASE VALUES
98	42	Gingiva	5.6	21.8	16.2	+7.3
99		Finger	5.9	14.8	8.9	
74	32	Gingiva	3.5	17.0	13.5	+7.9
75		Finger	3.5	9.1	5.6	
133	22	Gingiva	4.3	16.8	12.5	+8.1
134		Finger	4.7	9.1	4.4	
82	36	Gingiva	4.7	25.7	21.0	+8.3
83		Finger	6.0	18.7	12.7	
64	22	Gingiva	4.0	18.8	14.8	+8.4
65		Finger	5.2	11.6	6.4	
139	40	Gingiva	5.1	18.6	13.5	+8.7
140		Finger	4.9	9.6	4.8	
144	22	Gingiva	3.7	19.9	16.2	+10.6
145		Finger	6.3	10.2	3.9	
141	41	Gingiva	5.1	26.4	21.2	+11.0
142		Finger	4.3	14.5	10.2	
96	40	Gingiva	6.0	32.1	26.1	+11.8
97		Finger	5.4	19.7	14.2	
60	25	Gingiva	4.0	23.5	19.5	+14.2
61		Finger	3.9	9.2	5.3	
157	54	Gingiva	6.1	28.8	22.6	+14.2
158		Finger	5.9	14.3	8.4	
125	41	Gingiva	5.3	24.3	19.0	+14.2
127		Finger	6.8	11.6	4.8	
148	24	Gingiva	6.8	30.4	23.6	+14.4
149		Finger	4.7	13.9	9.2	
113	40	Gingiva	11.1	34.5	23.4	+14.6
114		Finger	11.8	20.6	8.8	
90	20	Gingiva	5.9	32.2	26.3	+18.1
91		Finger	5.2	13.4	8.2	
48	15	Gingiva	7.5	38.7	31.2	+22.3
49		Finger	6.6	15.5	8.9	

the gingival blood sample over the finger blood sample of +4.3 to +13.6 phosphatase units is shown; the mean difference is +6.6 units.

Table III in a similar way presents the phosphatase values in cases of radiographically indicated 'rapid' type of alveolar crest destruction. A further increase in the phosphatase concentration in the region of rapid bone loss seemingly occurred in this series of cases for the difference in phosphatase value ranges between +7.3 and +22.3 phosphatase units, with a mean difference of +12.1 units.

TABLE IV

Arrested phosphatase values in radiographically indicated alveolar crest dissolution

SPECIMEN NUMBER	AGE OF PATIENT	SOURCE OF SPECIMEN	ORIGINAL P UNITS	P UNITS AFTER INCUBATION	CALCULATED PHOSPHATASE	DIFFERENCE IN PHOSPHATASE VALUES
86	50	Gingiva	4.3	38.9	34.5	−2.7
87		Finger	4.0	41.2	37.2	
167	31	Gingiva	5.3	22.6	17.3	−2.0
168		Finger	4.7	24.0	19.3	
108	33	Gingiva	6.4	16.0	9.6	−1.2
109		Finger	7.9	18.7	10.8	
110	22	Gingiva	9.1	15.9	7.0	−0.3
112		Finger	9.9	17.2	7.3	
121	26	Gingiva	4.3	18.6	14.3	0.0
122		Finger	4.3	18.6	14.3	
159	31	Gingiva	9.2	24.2	15.0	+3.7
160		Finger	5.3	16.5	11.2	

Six cases of tests taken from interproximal regions that showed radiographic evidence of either 'slow' or 'rapid' alveolar bone loss but in which differences in phosphatase values between the gingival blood sample and the finger blood sample varied from the previous findings are presented in Table IV. Rather than an increased phosphatase value in the blood from the gingival region, it is evident that a decrease in phosphatase concentration occurred in the largest number of instances. The differences in phosphatase values, as calculated, range between −2.7 and +3.7 phosphatase units.

DISCUSSION OF PLASMA PHOSPHATASE FINDINGS FROM ALVEOLAR BONE TYPES

The ranges of plasma phosphatase differences between the unit findings of gingival blood plasma from the region of the alveolar crest and the values of the individual finger-prick blood plasma, show a significant variation toward a greater concentration of phosphatase in the plasma from the region of the radiographically indicated alveolar bone crest dissolution. In cases of normal alveolar bone crests the plasma phosphatase concentration in the region tends to be closely similar to the values found for the individual normal as determined by the plasma phosphatase of the finger blood. This tendency to approximate the phosphatase concentration found in the finger blood plasma is the expected finding, since with radiographically 'normal' alveolar bone structure, no pathosis is evident that might result in an increased phosphatase value. Only if such similarity were evident in the region of 'normal' alveolar crest bone would phosphatase increases in cases of alveolar dissolution be of significance. The determinations in the cases of 'normal' alveolar bone structure, shown in Table I, therefore represent a control group.

The average findings of the calculated phosphatase values from the finger blood plasma and the gingival blood plasma of the 'normal' and 'arrested' alveolar bone types combined, and the 'slow' and 'rapid' types of alveolar crest bone destruction, are represented in *fig. 1.* The mean or average plasma phosphatase values of the finger and gingival blood samples for the combined 'normal' and 'arrested' cases, show a close similarity. It is evident that the mean value in phosphatase units of the gingival blood samples is increased progressively from 12.6 units in the 'normal' alveolar bone gingival blood specimens, to 14.2 units in cases of 'slow' destruction, and reached a mean value of 20.0 units in the tests on 'rapid' type of alveolar bone crest dissolution. The higher mean finger blood plasma phosphatase value in cases of normal alveolar structure, in comparison to the finger blood plasma findings in cases of radiographically indicated 'slow' and 'rapid' alveolar crest destruction, is similar to the result in the smaller number of cases previously reported (3). The 'slow' type, with a value of 7.6 units, and the 'rapid' type, with a value of 7.8 units here indicate closer agreement.

Fig. 2 indicates the average unit difference of each of the 3 divisions of alveolar bone types. If the mean plasma phosphatase values of each type of bone are represented by such differences, a trend of increased plasma phosphatase concentration in the region of alveolar crest destruction becomes apparent. The difference increases from 0.6 phosphatase units in the normal alveolar crest type, to 6.6 units in the radiographically indicated 'slow' alveolar destruction, and reaches 12.1 units in the 'rapid' cases.

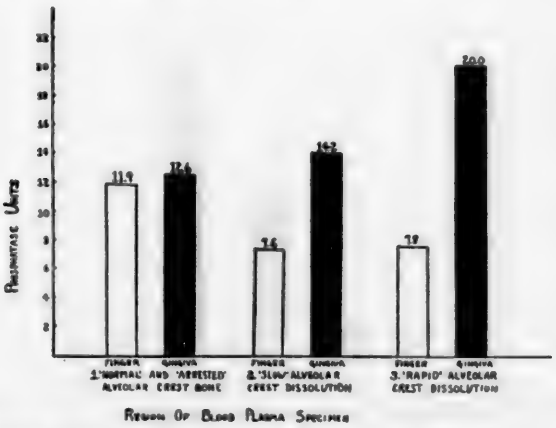

FIG. 1. Mean finger and gingival blood plasma phosphatase findings.

1. Mean phosphatase units found in finger-prick blood plasma and in gingival blood plasma from region of radiographically indicated 'normal' and 'arrested' alveolar bone by described method. 2. Mean phosphatase units found in finger-prick blood plasma and in gingival blood plasma from region of radiographically indicated 'slow' alveolar crest dissolution. 3. Mean phosphatase units found in finger-prick blood plasma and in gingival blood plasma from region of radiographically indicated 'rapid' alveolar crest dissolution.

A number of possible explanations may be suggested to explain the seeming deviation from this trend as presented by the cases in Table IV. These include: 1.—An error occurred either in the technique of sampling or in the determination. 2.—No change in plasma phosphatase values in the region of the alveolar crest dissolution occurred in the blood of the region, so yielding a resultant phosphatase value closely similar to that obtained from the subject's blood plasma. 3.—The blood sample from the interproximal area was taken too far from

the area of bone dissolution, so that dilution occurred from the unaffected superficial capillary blood of the gingival papilla. 4.—The original alveolar crest dissolution, indicated radiographically, was undergoing a change which may have been related to a time interval intervening between the taking of the radiograms and the plasma phosphatase determinations. 5.—A sufficiently active blood circulation existed in the gingival papilla, possibly in response to irritation or infection in the region, to cause no increased concentration of plasma

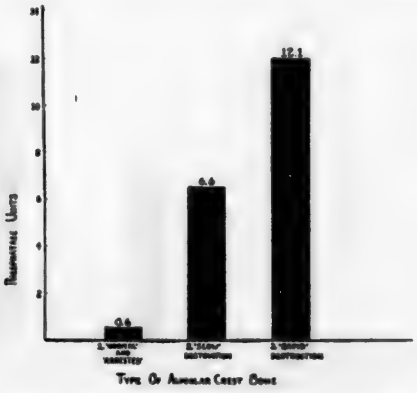

FIG. 2. Mean calculated differences in plasma phosphatase values.

1. Mean plasma phosphatase difference between plasma phosphatase units in region of radiographically indicated 'normal' and 'arrested' alveolar crest bone and finger-prick blood plasma. 2. Mean plasma phosphatase difference between plasma phosphatase units in region of radiographically indicated 'slow' alveolar crest dissolution and finger-prick blood plasma. 3. Mean plasma phosphatase difference between plasma phosphatase units in region of radiographically indicated 'rapid' alveolar crest dissolution and finger-prick blood plasma.

phosphatase in the region of the alveolar bone dissolution to become evident.

SUMMARY

1.—A micro-method for determination of plasma phosphatase value, adapted to use for gingival blood samples, is presented. 2.—Cases of radiographically indicated 'normal' and 'arrested' alveolar bone give a mean plasma phosphatase value of 11.9 units from the finger blood plasma, and 12.6 units from the blood plasma of the region

of the alveolar crest. 3.—Cases of radiographically indicated 'slow' alveolar crest bone destruction give a mean plasma phosphatase value of 7.6 units from the finger blood plasma, and 14.2 units from the blood plasma of the region of the alveolar crest. 4.—Cases of radiographically indicated 'rapid' alveolar crest bone dissolution give a mean plasma phosphatase value of 7.8 units from the finger blood plasma, and 20.0 units from the blood plasma of the region of the alveolar crest. 5.—The phosphatase differences of each of the 3 divisions of alveolar bone types was 0.6 units for the 'normal' and 'arrested' alveolar bone group, 6.6 units for the 'slow' alveolar crest destruction, and 12.1 units for the 'rapid' alveolar crest destruction. 6.—Six cases are presented in which no increased plasma phosphatase value was found in the region of alveolar bone crest destruction.

REFERENCES TO LITERATURE

(1) Gottlieb, B., *J. A. D. A.* **14**: 2178, 1927; *J. A. D. A.* **15**: 462, 1928.

(2) Miller, S. C., et al., *Textbook of Periodontia* (Philadelphia, P. Blakiston's Son and Co. Inc.: 1938), p. 144.

(3) Miller, S. C. and Pelzer, R. H., *J. A. D. A.* **26**: 565, 1939.

(4) Maitland, M., Hausman, F. S. and Robison, R., *Biochem. J.* **18**: 1152, 1924.

(5) Robison, R., *Significance of Phosphoric Esters in Metabolism* (New York, N. Y. U. Press: 1932).

(6) Kay, H. D., *Physiological Rev.* **12**: 384, 1932.

(7) Best, C. H. and Taylor, H. B., *Physiological Basis of Medical Practice* (Baltimore, William Wood and Co.: 1937), p. 1125.

(8) Roberts, W. M., *Brit. J. Exper. Path.* **11**: 90, 1930; *Brit. Med. J.* **1**: 734, 1933.

(9) Bodansky, A. and Jaffe, H. L., *Proc. Soc. Exptl. Biol. Med.* **31**: 1179, 1934.

(10) Duvoir, M., Layani, F. and Pantrat, J., *Bull. et mem. Soc. med. hop. de Paris* **51**: 1602, 1935.

(11) Bodansky, A. and Jaffe, H. L., *Arch. Int. Med.* **54**: 88, 1934.

(12) Labbé, G., *Revista Dental de Chile* **28**: 4, 1937.

(13) Bodansky, A., *J. Biol. Chem.* **120**: 167, 1937.

(14) Kay, H. D., *Brit. J. Exper. Path.* **10**: 253, 1929; *J. Biol. Chem.* **89**: 249, 1930.

(15) Lundsteen, E. and Vermehren, E., *Compt. rend. trav. lab. Carlsberg, Ser. chem.* **21**: 147, No. 10, 1936.

(16) Van Allen, C. M., *J. A. M. A.* **84**: 202, 1925; *J. Lab. and Clin. Med.* **10**: 1027, 1925.

THE TOXICITY AND RUBEFACIENT ACTION OF SULPHATED HIGHER ALCOHOLS[1]

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Soap detergents for certain purposes have two distinct disadvantages; they form insoluble metallic salts and they cannot be used in acid media. · Duncan (2) demonstrated that sodium salts of the higher sulphated alcohols possess most of the characteristics of an ideal "wetting out" agent and that their detergent properties are superior to soap, furthermore, that they can be used either in acid or alkaline solutions with equal efficiency. Provided that these higher sulphated alcohols are not toxic and can be tolerated by living tissues their use can be recommended in pharmaceutical preparations as well as in the textile and dye industries. They have been introduced into dentifrices, especially those with acid reaction or containing calcium salts, to replace soap (3).

Kitchin and Graham (4) tested a dentifrice made up with a 2 percent concentration of a commercial grade of alkyl sulphate. They concluded that this preparation had cleansing properties superior to that of soap with complete absence of tissue irritation. Other studies (5) have tended to substantiate their findings. Since there is some reason to believe that a slightly acid dentifrice is to be preferred and since many dentifrices contain calcium salts incompatible with soap, the toxicity and rubefacient action of the sulphated alcohols should be investigated, especially in so far as this concerns their incorporation into dentifrices.

Commercial preparations of the sulphated alcohols are on the market under such trade names as Duponal, Gardinol and Irium. Irium has been produced especially for use in a tooth paste and is mostly sodium lauryl sulphate with a small percentage of other

[1] This research was made possible through a grant from the Pepsodent Company.

sulphated alcohols of varying molecular weights. It was used exclusively in the following experiments.

Active albino rats, because they were small, inexpensive and easy to handle, were used. Young rats in good health and weighing not less than 150 gm. were divided into 3 groups made up of 25 animals each so selected that the total weight of each group was approximately the same. The standard diet throughout the experiment consisted of 2 parts of whole wheat flour, 1 part of powdered milk and 1 per cent iodized salt, to which was added 1 tablespoonful of canned white salmon once a week. Fresh water was given daily. The rats were fasted for 24 hours once each week, were kept in individual cages throughout the experiment, and were weighed at the beginning of the experiment and at the end of each week thereafter. After 3 weeks had elapsed, 30 milligrams of Irium was added each day to the food of the rats in Group 2, and 60 mgm. daily to that of the animals in Group 3; the rats in Group 1 were continued on the unchanged standard diet. The experiment was terminated at the end of the eighth week.

TABLE I

Weight changes recorded at weekly intervals

TIME IN WEEKS	GROUP 1—CONTROL			GROUP 2—LOW IRIUM			GROUP 3—HIGH IRIUM		
	Total weight	Gain	Loss	Total weight	Gain	Loss	Total weight	Gain	Loss
0	5870	—	—	5796	—	—	6440	—	—
1	6145	275	—	6135	339	—	6620	180	—
2	6487	342	—	6547	412	—	6932	310	—
3	6690	203	—	6687	140	—	7143	211	—
				Irium added			Irium added		
4	6638	—	52	6678	—	9	7292	49	—
5	6624	—	14	6706	28	—	7204	—	88
6	6567	—	58	6701	—	5	7296	82	—
7	6844	277	—	6877	176	—	7494	201	—
8	6853	12	—	6905	28	—	7565	68	—
Net gain....		985			1109			1013	

The weight changes are recorded in Table I and charted on graph paper as shown in *fig. 1.* It is significant that all animals not only survived the experiment but also all 3 groups registered net weight gains, indeed the net weight gains were greater for Groups 2 and 3, containing the animals that were fed Irium, than for the animals in Group 1, the control. The animals that received Irium did not differ in behavior and activity from those in the control group except that their feces were bulkier and more fluid. The total amount of Irium fed to each rat in Group 2 was 1,000 mgm. and to each one in Group 3,

2,000 mgm. This is equivalent to several times the amount contained either in tooth paste or powder as used in cleaning the teeth, very little of which of course is swallowed or ingested.

Four rats from each group were killed at the end of the experimental period. Careful dissection disclosed no gross changes in any of the organs, especially liver, kidney, stomach or intestine. Portions of these organs were fixed in formaldehyde and sections prepared and

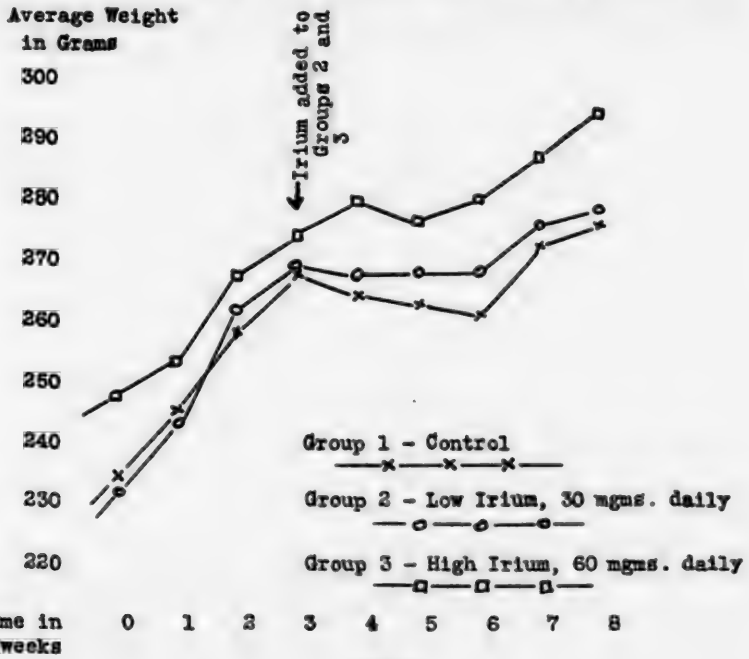

FIG. 1

stained with hematoxylin and eosin. Frozen sections were examined for fatty changes after staining with Sudan III.

All the kidney preparations are alike, there is no difference between the sections made from animals in the control group and those from the animals that ingested Irium. The liver cells of the animals that received the sulphated alcohol are somewhat swollen, the liver cords pressed together and the nuclei prominent; these changes are more noticeable in the livers of the animals that received the greater dosage of Irium. There is an increase in the number of cells between the gland crypts of the intestines of the rats fed the sulphated alcohol mixed with their food; these cells are mononuclears, many with a

marked affinity for eosin. Otherwise the liver and intestinal tissues of all groups are alike. There are no fatty changes disclosed in any of the livers and kidneys examined.

The dosage of Irium producing such changes amounted to more than 100 mgm. per kilogram of body weight. Comparable results for a person weighing 150 pounds would necessitate swallowing as much 20 ounces of Irium-containing tooth paste or powder, daily.

Most salts held in close contact with living tissues will produce some grade of irritation if the time of contact is long enough and the concentration great enough. In order to investigate the effects of Irium of this nature a modified "patch test" was devised; cotton

TABLE II

Sensory symptoms of irritation

TIME IN MINUTES	EFFECT OF 7½% SOAP SOLUTION	EFFECT OF 7½% IRIUM SOLUTION	EFFECT OF 3% IRIUM SOLUTION	EFFECT OF 2% IRIUM SOLUTION	EFFECT OF 1% IRIUM SOLUTION
0	−	−	−	−	−
1	−	−	−	−	−
2	−	−	−	−	−
3	−	−	−	−	−
4	−	+	−	−	−
5	+	+	−	−	−
6	+	+	+	−	−
7	+	++	+	−	−
8	+	++	+	−	−
9	+	++	+	−	−
10	++	++	+	+	−
15	++			+	−
20				+	+

Sensation unchanged −. Slight grade of irritation +. Marked grade of irritation ++. Where spaces are blank no tests were made for the indicated time and solution.

gauze was saturated with various solutions of the detergent and applied to the mucous membrane of the mouth at the muco-buccal fold of the lower molar region. Subjective sensory changes were recorded and the mucous surfaces were examined for visible signs of irritation after the patch was removed. At first the patches were held in place for definite times, later the gauze was maintained in position only until definite subjective symptoms of irritation were noted.

In all cases the sodium alkyl sulphate patch was placed against the mucous membrane on one side of the mouth and a patch wet with a 7½ per cent solution of powdered castile soap on the opposite side. Twenty-three subjects were used and the concentration of Irium varied, the percentages used being 7½, 3, 2 and 1.[2] At least 5 trials were made

[2] Irium concentrations materially greater than one per cent are supersaturated and the excess of Irium will precipitate out of solution on standing. These higher concentrations tested are, therefor, only of theoretical interest.

for each concentration. In Table II, the average time for the causation of irritation is compared with that for 7½ per cent soap solution. The degree of irritation is indicated by −, +, ++, the first indicating no irritation recorded, the second slight, the third definitely noticeable.

Since both the subjective and objective symptoms of irritation caused by soap and Irium differ qualitatively, a quantitative interpretation of the results of the above experiment as recorded in the table is only relatively accurate. The lesions caused by soap are not only hyperemic but the reaction seems to be definitely inflammatory in character, whereas in the case of Irium, although the superficial vessels are injected, the effect is similar to that following the use of an astringent. Subjectively soap causes a rather painful burning sensation which subsides rapidly after removal of the soap "patch". With Irium the sensory symptom is described as one of warmth rather than burning and this phase does not persist long after removal of the drug, however sensation in the region on which the "patch" was applied is different from that of the surrounding normal mucous membrane and this difference persists for a long time after the feeling of warmth has vanished. This sensation is similar to that following the use of an astringent gargle and the surface feels rough when the tongue is passed over it. This situation was considered to be a form of irritation and used to determine the duration of the effect of Irium.

Comparisons made on the above basis indicate that 7½ percent Irium solutions are definitely more irritating than 7½ percent soap, especially if the duration of the reactions are given due consideration, rather than the immediate degree of discomfort. The reactions to 3 percent Irium and 7½ percent soap were recorded as approximately alike with respect to the time of onset of disagreeable sensations, but the duration was greater with the Irium solution. It should be noted that one subject used in this group was highly resistant to the effects of soap solutions and reported no symptoms of irritation up to 11 minutes. The 2 percent Irium solution caused appreciably less irritation both subjectively and objectively than 7½ percent soap. This was even more striking in the comparison between 1 percent Irium and 7½ percent soap.

In connection with the above experiments it is well to bear in mind that soap is used in dentifrices in concentrations of from 5 to 16

percent, whereas the maximum concentration of Irium used in powder and paste is 1.25 percent.

SUMMARY

The continued ingestion of large amounts of sodium lauryl sulphate causes degenerative changes in the liver and intestinal mucosa of rats, but the amounts used were very greatly in excess of that found in tooth pastes and powders. The degree of surface irritation of human mucous membranes caused by this preparation is less than that of castile soap in the concentrations in which it is generally used. (Soap 5 to 16 percent; Irium, 1.25 percent.)

REFERENCES TO LITERATURE

(1) GRIFFITH, I., *Am. J. Pharm.* **106**: 176, 1934.
(2) DUNCAN, R. A., *Ind. and Eng. Chem.* **26**: 24, 1934.
(3) U. S. Patent 2,054,742.
(4) KITCHIN, P. C., AND GRAHAM, W. C., *J. A. D. A.* **24**: 36, 1937.
(5) RIDER, T. H., Private Communication.

THE EFFECT OF THYRO-PARATHYROIDECTOMY AT BIRTH AND AT 7 DAYS ON DENTAL AND SKELETAL DEVELOPMENT OF RATS[1]

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Thyro-parathyroidectomy of rats at birth results in a severe retardation of growth as judged by body weight (1), while those operated at 7 days show a much less severe retardation (2). It is the purpose of this paper to show the effect of the operation and, in a few cases, of subsequent parathyroid replacement on dental and skeletal development.

Of 678 rats operated by one of us (T. N. S.) on the first or second day of life, 240 survived the critical period of the first post-operative week, 64 of these being successfully thyroidectomized. This study includes 32 new born operated rats (6 of which received one unit parathyroid extract, Lilly, daily), 10 rats operated at 7 days and 31 unoperated normal controls, in most cases litter mates of the operated animals. The experimental animals, examined histologically for thyroid remnants, showed no trace of thyroid in all but 3 cases. These 3 have been included because there were less than 10 follicles and the animals showed no evidence of thyroid activity. The animals were sacrificed at intervals, skinned, eviscerated and their skeletons preserved in 95 per cent alcohol for several months before study.

The treatment of the dental material was as follows. Eruption time of the incisors was noted in the living animal, that of the molars was determined from fixed material. The term "beginning eruption time" as used herein designates the time when on examination of the maxilla, the cusps were first visible through the gingivae. Linear measurements were taken of the maxillary arch for length (the distance in mms. from the distal of the second molar to the gingival margin between the incisors) and width (the distance in mms. between the buccal surfaces of the second molars at the greatest dimension). One half the length times width was used in a comparative and statistical study of the maxillae. The molar teeth were measured in their natural position in the maxilla, after eruption of the anatomic crown. The mandibles were dissected out, radiographed, cleared of soft tissues, dried and weighed and the weights subjected to statistical analysis. The preservation in 95 per cent alcohol (several months) effected almost complete dehydration

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since a constant weight could be maintained after drying in air for one hour. The weights are comparable since all mandibles were treated in the same way. A report of the histological study is in preparation.

GENERAL FINDINGS

The systemic effect of thyro-parathyroidectomy of new born rats is a severe retardation of body growth (1). During the first week, their growth was only slightly below normal, but during the second, real retardation was apparent. After 3 weeks, body weight (about 25–30 grams) and skeletal development remained constant. The skeletal appearance did not progress beyond a stage corresponding to that of a normal rat aged 2 weeks (Table I). The epiphyses of the

TABLE I

The comparative development of control and operated male animals as indicated by data from representative individuals

AGE	UNOPERATED CONTROLS		THYRO-PARATHYROIDECTOMIZED								
			Newborn			Newborn—given I. U. parathyroid daily			7th day		
	Body weight	Body length	Body weight	Body length	Skeletal age	Body weight	Body length	Skeletal age	Body weight	Body length	Skeletal age
days	*grams*	*cm.*	*grams*	*cm.*		*grams*	*cm.*		*grams*	*cm.*	
7	12.8	6.9	9.2	6.5	7	9		7	13	7.1	7
14	25	8.6	17.2	7.2	9	18.5	7.3		24	8.3	
22	48	12.1	23	9.2	13	22.9	9.5	14	35	10.7	20
40	95	15.5	25	9.5	15	26.8	9.1	14	42	11.6	22
60	189	19.5	26.8	9.3	15	30	9.7	15	63	12.9	23

long bones remained small or did not ossify at all. The ventral rib segments remained cartilaginous. The skull proportions remained infantile, and the animals possessed symptoms widely ascribed to human cretins; i.e., sluggishness, lack of muscular coordination, intestinal stasis, and susceptibility to infection. The thymus was atrophied, the adrenal cortex was reduced, and the gonads remained infantile. With few exceptions, survival time did not exceed 60 days. Animals which did not eat well were given several feedings of milk daily with a dropper.

That the operation *per se* was not responsible for retarded growth was shown by complete removal of the thyro-parathyroid apparatus and re-implantation under the skin (3). Growth and behavior in such animals were normal.

Animals thyro-parathyroidectomized at 7 days of age showed less retardation than new born operated rats. Their growth curves showed a slow continuous rise (2). They were not as sluggish nor as helpless as those operated on the first or second day of life, appeared less susceptible to infection, and lived longer.

When a group of new born thyro-parathyroidectomized rats was injected with one unit of parathyroid extract (Lilly) daily, no growth response was elicited. Partial growth was obtained by feeding operated rats with dried thyroid powder, Armour, (0.1 to 1 mg. on alternate days). In still others the same dose of thyroid combined with one unit of parathyroid extract daily, produced normal growth (1).

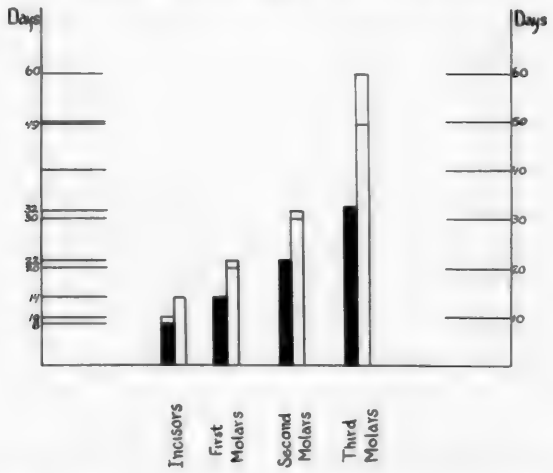

FIG. 1. Eruption time. ■ Controls. □ Experimental thyroid-parathyroidectomized rats operated first or second day of life.

DENTAL FINDINGS

1. *Beginning Eruption Time.* In animals operated on the first or second day, the incisors were first visible at 11–14 days, as compared with 8–10 days in the control group; the first molars at 20–22 days as compared with 14 days in the controls; the second molars at 30–32 days as compared with 21 days in the normals; and the third molars at 60 days (one at 50 days) as compared with 32 days in the normals (*fig. 1*).

2. *The Size of the Crowns* of the molar teeth (bucco-lingual measure-

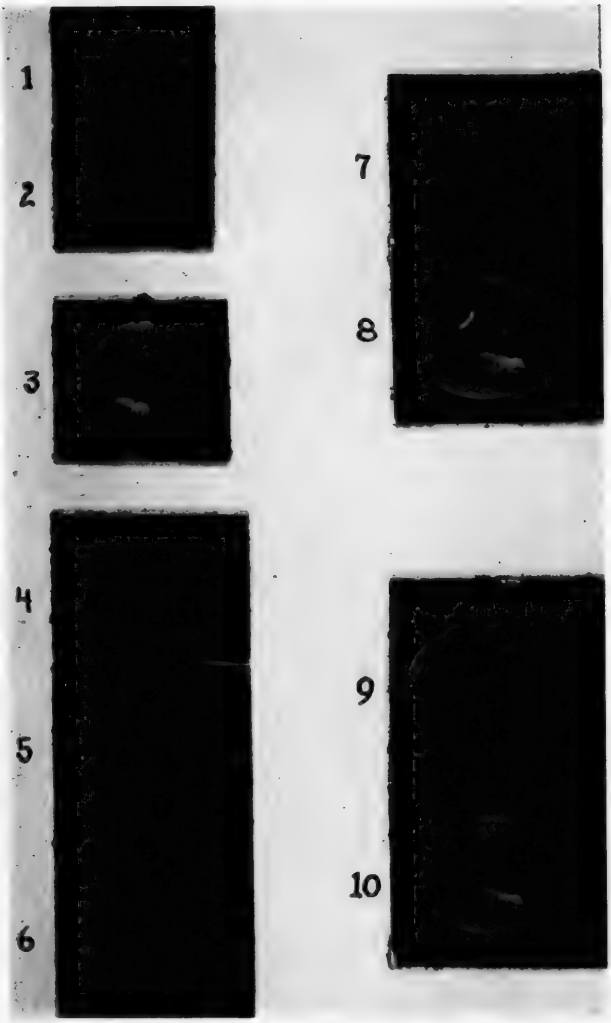

FIG. 2. Radiographs of rat mandibles. 1. Top ♀ Exp.; Bottom ♀ Normal; L. M Newborn thyro-parathyroidectomy. Sacrificed at age 14 days. 2. Top ♂ Normal; Bottom ♂ Exp. Newborn thyro-parathyroidectomy and 1 unit parathyroid replacement daily. Sacrificed at age 14 days. 3. Top ♂ Exp.; Bottom ♂ Normal; L. M. Newborn thyro-parathyroidectomy. Sacrificed at age 25 days. 4. Top ♂ Normal; Bottom ♂ Exp. Newborn thyro-parathyroidectomy. Sacrificed at age 40 days. 5. Top ♂ Exp.; Bottom ♂ Normal. Newborn thyro-parathyroidectomy and 1 unit parathyroid replacement daily. Sacrificed at age 40 days. 6. Top ♀ Normal; Bottom ♀ Exp.; L. M. 7th day thyro-parathyroidectomy. Sacrificed at age 40 days. 7. Top ♀ Exp.; Bottom ♀ Normal; L. M. Newborn thyro-parathyroidectomy. Sacrificed at age 61 days. 8. Top ♂ Exp.; Bottom ♂ Normal; L. M. Newborn thyro-parathyroidectomy and 1 unit parathyroid replacement daily. Sacrificed at age 60 days. 9. Top ♂ Normal; Bottom ♂ Exp.; L. M. 7th day thyro-parathyroidectomy. Sacrificed at age 70 days. 10. Top ♂ Exp.; Bottom ♂ Normal; L. M. 7th day thyro-parathyroidectomy. Sacrificed at age 85 days.

ments) was the same in the operated animals as in the unoperated normal controls. There was, however, marked retardation of root development in the molar teeth of the operated animals (*figs. 2, 3, 4*).

FIG. 3

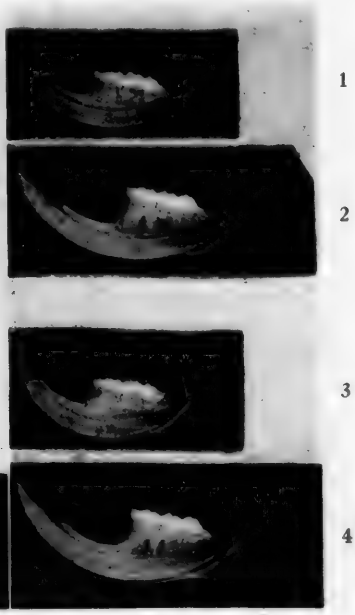

FIG. 4

FIG. 3. Radiographs of rat mandibles enlarged one and one-half diameters. 1. ♀ Exp.; Newborn thyro-parathyroidectomy; Sacrificed at age 22 days. 2. ♀ Normal control; Sacrificed at age 22 days. 3. ♀ Exp.; Thyro-parathyroidectomy on 7th day; Sacrificed at age 40 days. 4. ♀ Exp.; Newborn thyro-parathyroidectomy; Sacrificed at age 40 days. 5. ♀ Normal control; Sacrificed at age 40 days.

FIG. 4. Radiographs of rat mandibles enlarged one and one-half diameters. 1. ♀ Exp.; Newborn thyro-parathyroidectomy; Sacrificed at age 61 days. 2. ♀ Normal control; litter-mate of #6; Sacrificed at age 61 days. 3. ♀ Exp.; Newborn thyro-parathyroidectomy; Sacrificed at age 71 days. 4. ♀ Normal control; litter-mate of #8; Sacrificed at age 71 days.

3. *The Weights of the Mandibles* of the 3 series were plotted against age and since the points of each series appear to fall on a straight line, they were fitted with regression lines of the type $y = bx + a$ by the method of least squares. In this equation, y is the weight of the mandibles in milligrams, x is the age in days, and b is the slope

of the line or the daily increment of growth. The value of the slope of the lines for the operated series as compared with their normal controls gives a measure of the retardation of the development of the jaws caused by the operation. The equations of the regression lines and the slopes of these lines with their standard deviations are given in Table II. We have also included the standard error of estimate as a measure of the scatter about the regression line. The curves are shown in *fig. 5*.

TABLE II

Weights of mandibles

	CONTROLS	OPERATED	
		7 day	New born
Regression equation	$y = 3.75\,x - 6.2$	$y = 1.74\,x + 34.4$	$y = 1.87\,x + 6.9$
Slope of trend, mgm. daily increment	$3.75 \pm .22$	$1.74 \pm .26$	$1.87 \pm .098$
Standard error of estimate	18.02	12.8	8.58
Correlation coefficient	$.970 \pm .109$*	$.919 \pm .055$*	$.962 \pm .014$*

* Formula for σ of correlation coefficient $= \frac{1 - r^2}{\sqrt{n - 2}}$.

TABLE III

Indicated differences in weights of mandibles

	MGM. DAILY INCREMENT—DIFFERENCE
Control vs. new born operated	$3.75 - 1.87 = 1.88 \pm .199$
Control vs. 7 day operated	$3.75 - 1.74 = 2.01 \pm .312$
New born operated vs. 7 day operated	$1.87 - 1.74 = 0.13 \pm .278$

The chief interest in the present experiment lies in the observed differences in the average daily increment of growth of the 3 series (slope of the regression line) (Table III).

Both the new born and seventh day operated series when compared to the normal controls show an average daily increment of approximately one-half that of the normals. Although the absolute weights of the mandibles of animals operated on the 7th day exceeded those of animals operated on the first or second day, there is no apparent difference in the daily increment of the 2 series after the operation.

There is an interval between the operation and any noticeable effects, so that animals operated at 7 days have an initial advantage.

4. *The Determination of the Rate of Development of the Maxillae.* The area of the jaw was represented by one-half the product of the length of the dental arch by its width (triangle). This represents the combination of 2 variables in a single and significant value. The data were fitted with lines by the method of least squares. In the

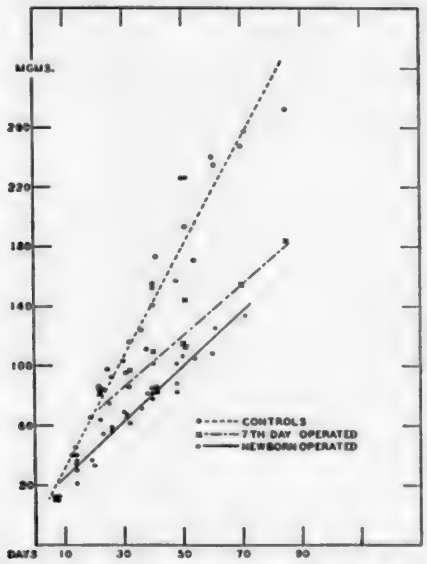

FIG. 5. Weights of mandibles of 3 groups of rats, dried for one hour after alcohol dehydration. Group 1: 31 animals, thyro-parathyroidectomized on first or second day of life. 6 of these received 1 unit parathyroid extract replacement daily, apparently without effect on growth, or on the development of the teeth. Group 2: 10 animals, thyro-parathyroidectomized on the seventh day of life. Group 3: 31 normal, unoperated controls, in most cases littermates of animals in Groups 1 and 2.

formula, $y = bx + a$, y represents the area in square mms. of the dental arch and all the other variables have the same definition as in the formula used for the mandibles. The statistical constants of the area of the dental arch are given in Tables IV and V, the graphs in *fig. 6*.

The animals operated on the seventh day of life show an average daily increment in the area of the dental arch of approximately one-

TABLE IV

Area of the dental arch

	CONTROLS	OPERATED	
		7 day	New born
Regression equation.....	y = .6029 x +42.2	y = .3308 x +43.3	y = .1872 x +45.2
Slope of trend, sq. mm. daily increment.....	.6029 ±.056	.3308 ±.081	.1872 ±.058
Standard error of estimate.....	3.608	3.36	2.201
Correlation coefficient...	.927 ±.03*	.838 ±.113*	.665 ±.155*

* Formula for σ of correlation coefficient $= \frac{1 - r^2}{\sqrt{n - 2}}$.

TABLE V

Indicated differences in area of the dental arch

	SQ. MM. DAILY INCREMENT—DIFFERENCE
Control vs. new born operated.....	.6029 − .1872 = .4157 ±.081
Control vs. 7 day operated.....	.6029 − .3308 = .2721 ±.098
7 day operated vs. new born operated.....	.3308 − .1872 = .1436 ±.099

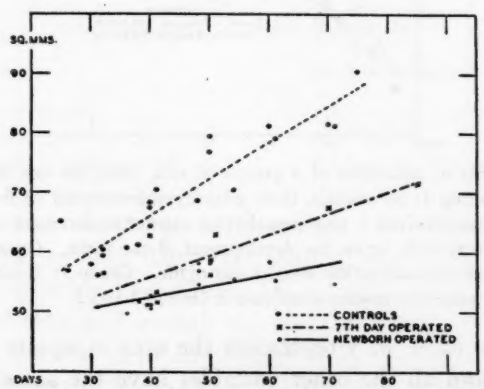

FIG. 6. Measurements expressed in square millimeters of one-half length times width of dental arch. Group 1: 15 animals, thyro-parathyroidectomized on the first or second day of life. Group 2: 9 animals, thyro-parathyroidectimized on the seventh day of life. Group 3: 21 normal, unoperated controls, in most cases littermates of animals in Groups 1 and 2.

half that of the normal controls. The corresponding growth rate of the new born operated rats was about one-third that of the controls. The average daily increment in the new born series was less than that of the seventh day operated group but the figures show that this difference is less than twice its standard deviation and therefore lacks statistical significance. We feel that if a larger number of animals had been included in the present study we might have found a real difference in the growth rate of these 2 series. This assumption

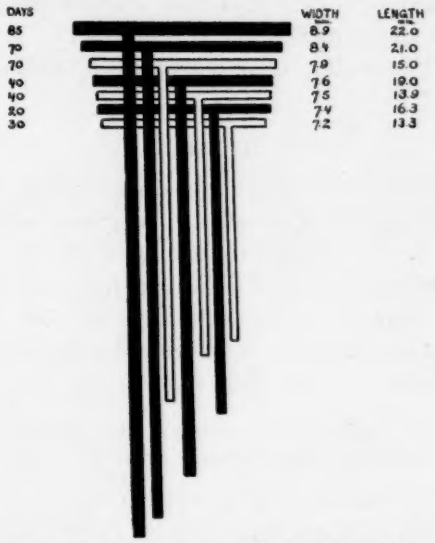

FIG. 7. A comparison of the length and width of the upper maxillae of thyro-parathyroidectomized rats operated on the first or second day of life and their controls. □ Experimental. ■ Control.

is based on the knowledge that body, skeletal and organ growth rates (the latter unpublished data) are less retarded in the 7 day operated than in the new born operated series.

DISCUSSION

From the gross observations of material here presented, it is obvious that thyro-parathyroidectomy causes marked retardation in growth and development (Table I). It is noteworthy, however, that while body growth of new born operated animals remains constant

after about 3 weeks, the jaws progress in subnormal development (observations limited to the duration of this experiment—71 days). The administration of parathyroid extract to the new born operated animals caused no difference in either their body or dental development.

The greatest retardation occurs in the length of the jaws rather than in the width (*fig. 7*). This is probably due to the effect of the deficiency caused by ablation on the continuously growing incisors (*fig. 2*). It is also significant that while there is retardation of molar dentin and root development, the size of the crowns, apparently is not affected. (See enlarged photographs of radiographs, *figs. 3 and 4*).

CONCLUSIONS

1. Early thyro-parathyroidectomy causes severe retardation of growth and development generally. There is a retardation of (a) about one-half in the weights of the mandibles of new born and 7th day operated animals; and (b) of one-half (7th day series) and two-thirds (new born series) in the area of the dental arch.

2. While eruption time, dentin and root development are retarded, the size of the molar crowns remains unaffected.

We acknowledge with gratitude the suggestions of Professor Earle Phelps of Columbia University, and his supervision of the statistical analyses.

REFERENCES TO LITERATURE

(1) SALMON, T. N., *Endoc.*, 23: 446, 1938.
(2) *Idem, Proc. Soc. Exp. Biol. & Med.*, 35: 489, 1936.
(3) SALMON, T. N., AND SEVERINGHAUS, A., *Proc. Soc. Exp. Biol. & Med.*, 34: 251, 1936.